BIOBSERVE
SOLUTIONS FOR YOUR SCIENCE

Viewer^3

User Manual

The VERSATILE software for VIDEO TRACKING systems

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Viewer^3
Video Tracking Software

Thank you for purchasing the *Viewer* and congratulations to your decision. The *Viewer* software was developed by biologist and is the ultimate tracking system. Into the bargain the developers gave a sophisticated statistical analysis that delivers an outright idea of the gathered data – even during acquisition. BIOBSERVE has the longest three point-tracking experience and hence can offer most advanced behavior recognition features.

The *Viewer* program is composed of three succeeding modes, reflecting the work steps when an experiment is executed: Configuration, Acquisition and Data analysis. In the same way this manual is built up, except that there is a last chapter, dealing with main menu items.
In the manual you will find a lot of words and phrases in italic letters. This kind of notation indicates that the used words exist as labels in the program interface or represent functional program components.

To train your skills in handling the *Viewer* equipped laboratory, we advice to work through the manual while testing on a video recorded film. To get a free digital video please contact

support@biobserve.com

Installation

The *Viewer* software runs on PCs and MAC computers with Windows operating system. This includes MAC Windows emulations. The way how to start the installation depends on the storage medium the program was delivered on. A CD will initiate the installation process by itself when inserted. If your CD-drive is not configured to detect autoplay volumes (most drives are), in any case installation can be started manually. Just double click the *setup.exe* file. A wizard will lead you through the whole process, thereby proposing the following program folder location.

c:\Program files\BIOBSERVE\Viewer (c:\ is a placeholder for your operating system drive)

If you want to determine the software location on your hard drive by yourself, you should create an appropriate folder in advance. The installation wizard gives the opportunity to browse there to indicate it as new destination for installation. In the program folder all necessary files and sub-directories are generated automatically.

Updates

Viewer updates are delivered for free, until there is a major version development. Unlike other programs *Viewer* has no internet based update check, because most laboratory computers are not connected to the internet permanently. The easiest way to stay informed is to subscribe to our email newsletter or to visit our homepage and read the latest news frequently under

http://www.biobserve.com/company/news.html

Updating is exceptional simple and quickly done. Updates with all topical new features are delivered as *viewer.exe* file. Place the new file in the program folder – and that's it. To be on the save side it is recommended to backup the old *viewer.exe* before updating. Otherwise it will be replaced. Rename it or copy it to another folder. Of course, configurations and results survive updating.

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Configuration

The first step is to create the configuration. That means setting up the parameters for data recording and processing. Click the Configuration tab to access the program's *Configuration* mode, resp. page. The software's *Configuration* page itself is divided into seven subordinated tabs. In *Experiment* you can type in a description of the experiment as well as adjust the settings to start and stop the experiment automatically. The *Camera* section enables you to configure the camera that you will use, while *Filters and Objects* allows you to adjust the filter in order to detect the animals in the experiment arena. On the *Zone Definition* tab you can define different zones within the arena which will be evaluated separately in the subsequent data acquisition. In *Activity* you can determine velocity classes and activity thresholds. On the *Behavior* tab, filters resp. thresholds for the detection of different behaviors can be adjusted. At last the *Saving Options* tab allows you to define video recording settings and the length of saving intervals, necessary for obtaining interim data results. When you start the software the first time, a sample configuration is displayed. The name of the current configuration will be indicated at the top on the right side.

Configuration files

Save configuration

You can always save settings to use them again later. The eligible commands are located in the *Configuration* menu (see chapter *Program main menu*) and the vertical configuration menu bar on the left of each page under the *Configuration* tab. Select either *Save configuration* or *Save configuration as...* (third icon from the top) to save it in a new file with a new name. *Viewer* configuration files (*.vcf) influence the interpretation of raw data and thus should be kept to produce comparable data or to perform recalculation on data. Configuration files will be saved in the *Config* folder within the *Viewer* program folder, unless you choose another place.

figure 1

Open configuration

In case you would like to use an already existing configuration file to carry out new experiments with, you can open the file from the *Configuration* menu by selecting *Open configuration*. You can also use the analog speed buttons in the vertical task bar (uppermost icon).

Export configuration

By selecting *Export Configuration* (in the *Configuration* menu, page 56) you can produce a printable version of the configuration with all its information about individual settings.

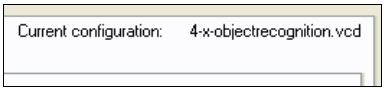


figure 2

Configuration | Acquisition | Data analysis

Experiment | Camera | Filters and Objects | Zone Definition | Activity | Behavior | Saving Options

Experiment

Description – Time controlled Execution – General Trigger Control

On the *Experiment* labeled tab you have the opportunity to type in a description of the experiment. This description will later be saved as meta data in your result exports as well. If you want to start and stop an experiment automatically, you can enter start and stop times here. Time-dependent triggering of an output-channel may be adjusted, too.

Descriptive fields

There are several text boxes where you can type in information that describes the experiment. All the information is saved, together with the raw data, in the *Viewer* result files. You can name the experimenter (*Experimenter*) assign a number (*Experiment No.*), describe the experiment in a few words (*Short description*, useful for keyword search), and more words (*Long description*). You can name the test animal (*Animal name*) and assign a number (*Animal No.*) to it. How to name multiple animals in one setup will be discussed in chapter *Configuration − Filters and Objects − Tracking area*.

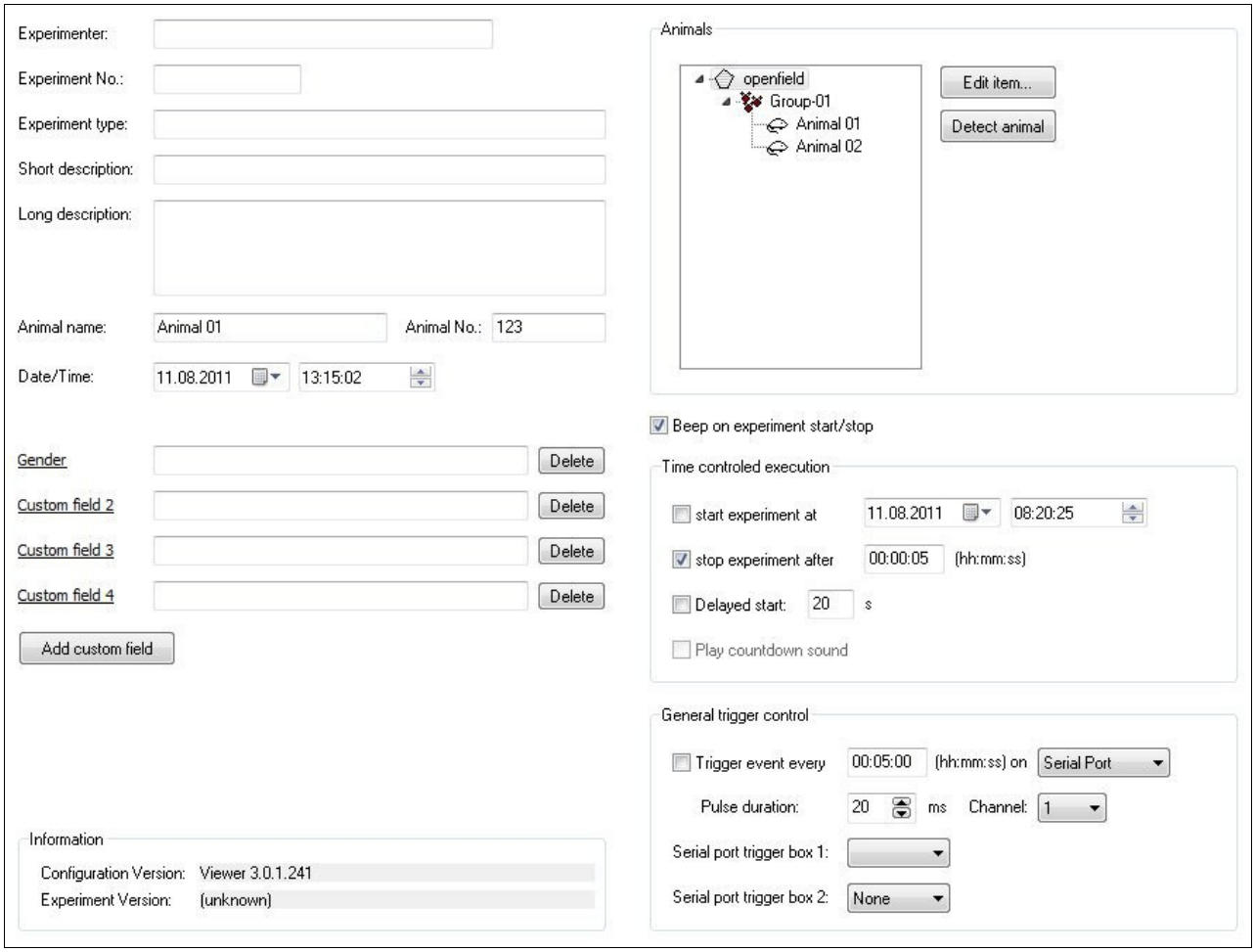


figure 3

Custom fields

Besides the predefined text fields, there are customizable fields. Their default names can be changed. To do so, click the underlined *Custom field* label. A pop up window occurs where you enter the new name. The first *Custom field* name in figure 3 is already adapted (*Gender*). The names for these fields will be saved in the *Viewer* configuration, so you can use them again in your next experiment. To add a *Custom field*, operate the *Add custom field* button below the fields. To remove a special field, use the *Delete* button behind it.

Start and stop sound

Under the *Animals* labeled box on the right side is a single check box: *Beep on experiment start/stop*.
If the box is ticked, Viewer will emit a sound on acquisition start and stop. Such a sound can be helpful as feedback if you start acquisition with a remote control. On the other hand the sound can confuse or stimulate the test animal, if your *Viewer* computer is located very near to the experimental setup.

Animals info box

On the right side of the vertical separator in figure 3, there is an *Area* titled box. By default one area, one group and one animal are depicted there in a tree view. The display mirrors the *Area* settings. Additional items can be defined later on, as we will see in chapter *Configuration - Filters and Objects*. But the box is not solely a display, some attributes can be edited here as well.

Edit item

On the right from the *Animals* info box there are two buttons. *Edit item...*and *Detect animal*. Both relate to the content in the box. The *Edit item...* button is used to change an area's, a group's or an animal's name – depending on the item selected in the tree in the *Animals* info box. In case an animal is selected, the pop-up window in figure 4 opens when the button is operated. Here you can enter a new name and select a color for the animal's *Reference points* (see chapter *Zones*, page 25) and tracks. Especially in social interaction tests it can be quite useful to define different track colors to facilitate differentiation.

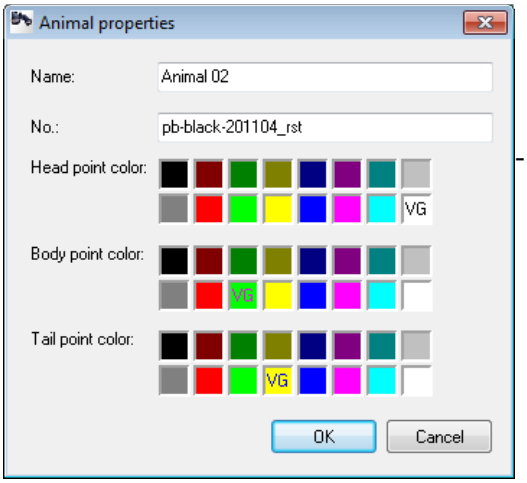


figure 4

Detect animal

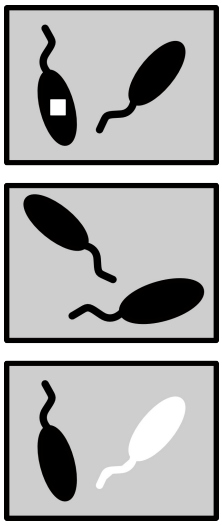


figure 5

Each animal tracked in an arena has a *Viewer* internal ID. The ID is associated with the animal's data. During acquisition it denotes the animal's body point and finally it headlines the result table. Contrary to the animal name, that might be long, expressive or similar for several animals at the same time, an ID is essential and unique. That's necessary, for example, to distinguish between animals in the same arena. Therefore it is allocated automatically. By default the program consults the configuration (see chapter *Filters and Objects*) for the number of animals and then numbers the animals consecutively from ID 1 to ID n. These IDs are displayed in the *Animals* info box (figure 3). The *Detect animal* button offers an alternative way of identification.
Primarily it was created to simplify result table naming and recognition in tests with two different animals. As soon as the area and the animal detection filters are configured (see chapter *Filters and Objects*) the software is able to identify the animals. Put or leave the animals in the arena, or playback your video. Then operate the *Detect animal* button. According to special characteristics, the animals are named automatically (figure 5, top-down):
Tagged and *Pristine*, *Small* and *Big* or *Dark* and *Light*

Time controlled execution

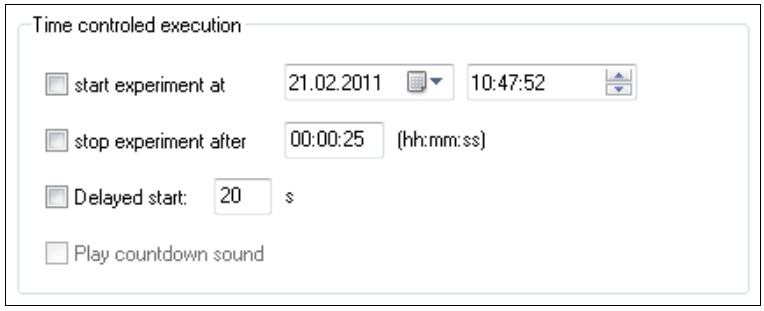


figure 6

Recording date and time of the experiment is done automatically. The displayed *Date/Time* values refer to the last accomplished experiment. They will be updated in the moment the next acquisition is started.
If you would like to set up the software for an automated, time controlled acquisition, you can do so in the *Time controlled execution* field by defining the date, the start and the stop time. To set the start time select the *Start experiment at* option and then choose the time and date for the next experiment by clicking the corresponding arrows in the date and time boxes. A drop-down calendar is attached to the date box. It might be more comfortable to pick the date from the calendar sheet than to enter it manually.
To stop the experiment automatically after a certain duration, select the *Stop experiment after* option and then enter the hours, minutes and seconds manually. This is an further function to fully automate experiments where the experimenter doesn't needs to be attendant.
A delayed start can be sensible when you work alone in your laboratory without the *Remote control* plug-in (see attached plug-in list). Start the experiment (see chapter *Acquisition*) before you go to the cages to put in the animals. Acquisition will not start until the time elapsed that you entered in the field *Delayed start:* [ ]. A small pop-up window displays the countdown. To hear the countdown (up to 20 seconds) check the *Play countdown sound* check box additionally.

General trigger control

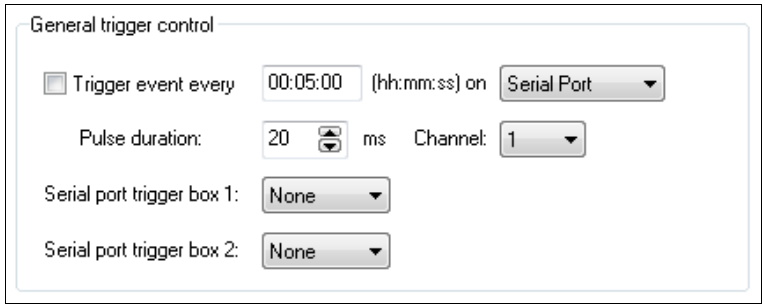


figure 7

Output-channel control by the *Viewer* may be used to control external devices during an experiment. For example food dispensers, stimulus devices, doors etc.
Trigger settings in the *Viewer* can be time-dependent and event-dependent. *General trigger control* options concern time-dependent settings and BIOBSERVE *Trigger box* settings.
If you want to send out a trigger signal automatically every X minutes, activate the *Trigger event every* [ ] check box, and enter the desired time interval. Timeformat is

hours:minutes:seconds.

Select a *Serial Port* or *Parallel Port* from the drop-down menu on the right from the time input field. In case you selected the *Parallel Port* you can determine a channel from 1 to 8 in the *Channel* drop-down menu below the port drop-down.
The *Pulse duration* input field defines the duration of the trigger signal in milliseconds. This is especially useful if you directly control a light, sound or shock device.
The two *Serial port trigger box #* drop-downs have to be used to define the COM port of the respective trigger box. Once the port is set, you can use trigger options for zone visit related triggers, as we will see in chapter *Zone definition*.

Information

This box simply informs about which *Viewer* version saved the currently used configuration and result file (*Experiment*).

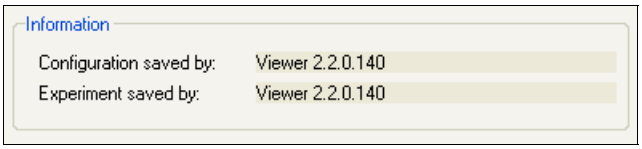
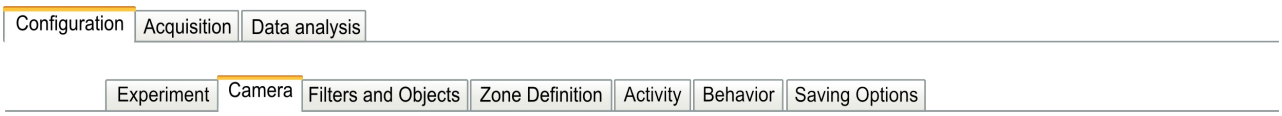


figure 8



Camera

Video Settings – Movie File

Video settings

Start the *Viewer* after the camera has been connected and turned on. You will find the models of all available video sources in the *Video settings* field. If several cameras are connected to the PC, click the arrow in the *Video devices* drop-down box to pick the device you want to use from the list.

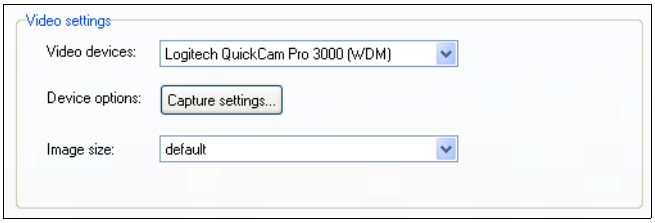


figure 9

Note: It is important that the camera is connected to the computer and turned on before you start the *Viewer*. Otherwise the camera can not be detected by the program and the message “No video device found” will be displayed when you start the software. If you switch on the camera under Microsoft Windows, the operating system will prompt a message within a short time, stating that a video device was detected. Cancel the MS Windows dialog to avoid the system to run Windows Movie Maker to record films.

You have the possibility to define *Capture settings*. The adjustable parameters depend on the connected camera and the installed capture-card. If you are not sure, maintain the standard settings.
With the *Image size* pull down menu you can determine the size of the video image used in the *Viewer* software. These settings effect the frame rate of the computer. The bigger the size of the display chosen, the slower is the frame rate.
As far as the camera supports resizing, the options listed in the table on the right are available. We suggest to use full resolution.

image size table	NTSC	PAL
Full	720 x 480	720 x 576
Half	360 x 240	360 x 288
Quarter	180 x 120	180 x 144
One-Eighth	88 x 66	88 x 72

Movie file

If you want the software to analyze a movie file of your experiment instead of a live video, click *Open...* and select the appropriate file in the *open* dialog. Once you have loaded a file, the name and path is shown behind the *File name* label. Whenever you see a displayed file name, it is possible to switch from life signal to movie file by activating and deactivating the *Use movie* check box.
In case your computer doesn't run proper during acquisition due to lacking processing power, you might reduce the frame rate in the *analyze every* [ ] *frame* field. The frame rate specifies the amount of pictures (frames) per second (fps) of a digital video.
Example: Analyzing an 18 fps video with *analyze every* [2] *frame* setting, will deliver 9 data points per second.

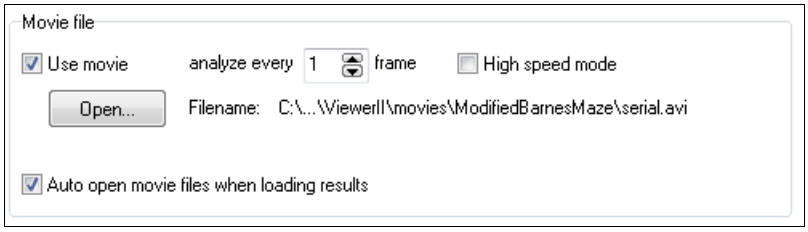


figure 10

In standard analyzing mode *Viewer* can slow down movie playback speed to process a huge amount of data in each frame (for example in social contact experiments). If there is enough processing capacity, the software plays back videos and computes everything in real time. The *High speed mode* offers the opportunity to accelerate video file analyzing. With this mode activated *Viewer* analyzes frame by frame as fast at it can. The video playback speed is then determined by the computer's processing speed.

The check box *Auto open movie files when loading results* becomes relevant when reviewing already acquired. After acquisition *Viewer* saved a video file with the same name as the result file in the same folder. With this check box activated *Viewer* opens these similar named videos from the same folder, when opening a result file.

By the way: If you save similar named configuration and video files in the same directory, *Viewer* also asks to load the configuration when the video is loaded (figure 11)

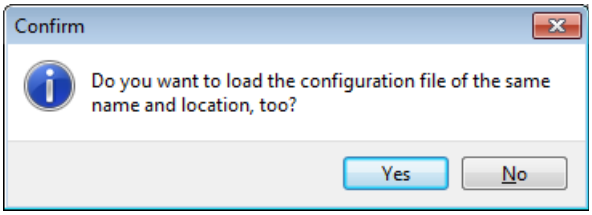


figure 11

Filters and Objects

Live Picture / Filtered Picture – Setup Calibration – Tracking Mode – Tracking Area – Background Filter
Animal Recognition Settings – Animal Filter – Display Settings

Live picture / Filtered picture

If a camera is connected to the PC, you will see the live picture in the *Live picture* box (figure 12). With the *Switch On* and *Switch Off* buttons the camera can be remote controlled to provide or not to have a picture in the *Filtered picture* box on the right side. Based on this picture the filters are adjusted. It is displayed with the resolution you selected in the previous section: *Camera*.

figure 12

Note: If you use a movie file you do not need to operate the *Switch On* button because the movie file appears in the *Filtered picture* display. Underneath it there is a toolbar with a regular movie control panel. Press the *Play* button to get an image, so you can define the tracking area and filters.

Setup calibration

Setup calibration is necessary to produce comprehensive values regarding distances. It enables the software to convert pixels into real world units. Calibrate before defining tracking areas and zones!
To calibrate the *Filtered picture* put a rectangular object of known size into the arena. A rectangular sheet of paper for example. Click *Calibrate* and move the cursor over the video picture. The cursor now appears as a cross with a ruler symbol. Press the left mouse button on the object's corner and hold it to skirt the object. Of course you can outline the whole arena as well. This is even recommended to reduce wrong measurement caused by the camera's lens distortion effect.

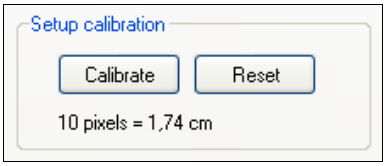


figure 13

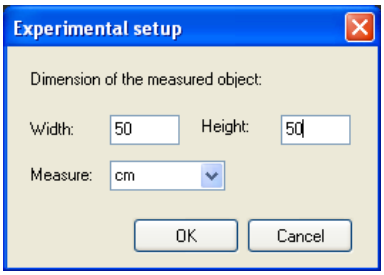


figure 14

Note: In any case it is necessary to calibrate on the arena's ground level and to center the calibration rectangle in the image.

When the mouse button is released a window pops up where you enter the width and length of the object. The selected unit will be relevant for all statistics later on. Use a point to separate decimals when running an English operating system. Otherwise use a comma. Now the calculated factor is displayed under the *Setup calibration* buttons and the *Filtered picture* is contorted to fit the new and compulsory dimensions. Contortion can be minimized by an absolutely perpendicular camera position above the experimental arena and precise drawing of the calibration rectangle. The *Reset* button removes the calibration.

Tracking area

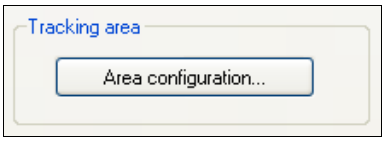


figure 15

Before adjusting any animal recognition settings, at least one tracking *Area* has to be created to inform the software where to search for animals.
In a result file the animal's track is inseparable associated with the tracking *Area*. You can compare the track to a graph and the area to the coordinate system the graph lies in. Thus it is not possible to change tracking *Area* settings after data acquisition. The pop-up warning in figure 16 explains, that you may change the *Area* configuration, but you can not apply it to an existing track.

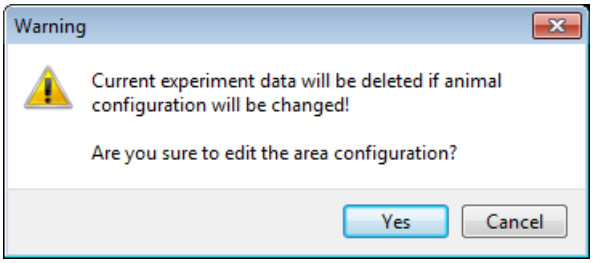

Warning
Current experiment data will be deleted if animal configuration will be changed!
Are you sure to edit the area configuration?
Yes
Cancel

figure 16

There are great benefits in working with tracking *Areas* compared to analyzing the whole video. First of all it is not necessary to process all pixels the camera delivers.
And often it is not possible to provide a perfect background in the whole video image. With *Areas* this is not necessary. The *Area(s)* within the video image fit exactly the experimental arena(s). So it doesn't matters if you see somebody in the video running around the setup, as long as nothing crosses or shades the tracking *Area*. Press the *Area configuration...* button to go on (figure 15).

In the *Tracking areas* window you can draw one or several separate tracking *Areas*. The software produces one experiment result table per *Area*. That means, if two open field boxes are observed simultaneously, it is necessary to create two tracking areas. Although it is technically possible to define only one area that covers two or more setups, such a configuration would mix up your statistics later on.

Activate one of the buttons *Rectangle*, *Ellipse* or *Free formed* to draw a tracking area. When one of these buttons is locked, the cursor becomes a drawing cross as soon as it is moved over the video image.

In figure 17 the dark gray background represents the video image. With the *Tool color* buttons (at the bottom of the vertical tool bar) you can preselect the line color to be black or white.

With the *Free formed* tool you can gird a any shape. A free formed area is created by clicking points. Lines straddle from one clicked point to the next. The shape is finished automatically when you place a point very near to the starting point.
With the cross-cursor tool (second from the top, below the arrow tool) you can reposition single points of a *Free formed* area by dragging them to change the area's shape.

Type and size of each tracking area appears in the table below the image automatically. To modify a completed area, select an area from the table in this window by clicking into the respective table line or click the area's rim with the pointer tool. Then use the square handles around the selected area to change its size and shape.

In the table you can enter a name for each tracking area. This name will later be used to title the result tables (figure 79). To delete an area, select the zone in the table and press the *Delete* button.

The table on the right from the video holds data size and position of the currently chosen area. The values in the table can be edited and used to align and to size areas precisely.

Experiment | Camera | Filters and Objects | Zone Definition | Activity | Behavior | Saving Options

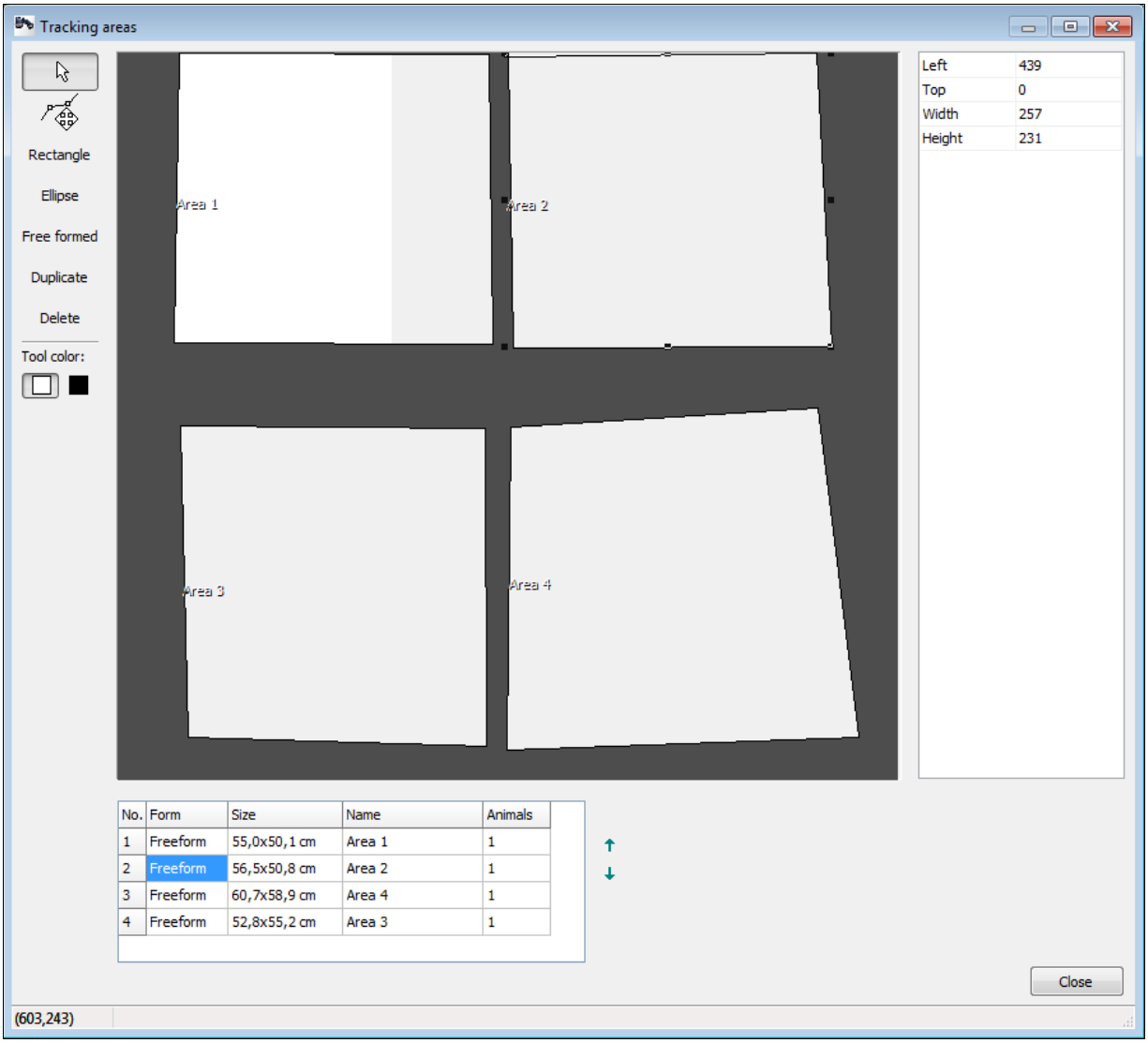


figure 17

Note: Define one area for each experimental setup! Do not let areas overlap!

Tracking several areas at the same time

It is possible to track and analyze several experiments at the same time. The *Duplicate* button may be used to duplicate a selected area. If you observe four similar boxes, for example, you can draw one area around a box and copy it four times. Finally the similar areas have to be arranged on the screen and and named in the area table.

It is not allowed to build areas that include, exclude or overlap other areas. Tracks that lie in more than one area produce data in both. This rule has to be kept in mind, because it is different from the way overlapping zones are handled by the software. Although the drawing tools are more or less the same.

As an area is a place in the video image where the software shall search for an animal, the program expects to find at least one animal in each area. That is why the *Animals* field in the *Tracking areas table* always contains the cypher 1 as default setting when an area is added. If you want to track more than one animal in an area, click into the field in the *Animals* column. Another pop-up window appears (figure 18) where animals can be added and grouped, which is some times necessary (see next chapter).

Tracking several animals at the same time

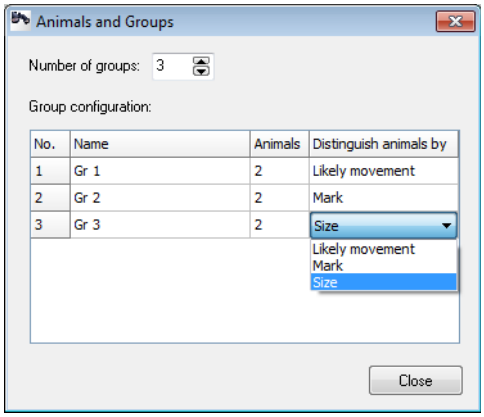


figure 18

Maintaining the right identities is fundamental for a reasonable interpretation of data from more than one animal. The initial ID of an animal is simply determined by the chronological order of first detection. The animal that is nearer to the zero/zero coordinate in the upper left corner will be detected first and will receive ID-1.
In experiments with two or more individuals animals often touch each other. Their silhouettes merge to a single pixel mass that can not be interpreted as animal shape anymore. When they separate again, the question comes up: which one was which? Even for a human observer it can be difficult to make out the correct animals, when similar colored rodents bustle around.
If all animals are colored alike the software re-establishes IDs if necessary according to the algorithm called *Likely movement*. It is assumed that animals leave each other in the direction they came from and that they do not climb over each other and preserver their direction of movement. The software can use additional algorithms to maintain correct IDs: It can focus on size, color or on marker (see figure 5).
To make these routines work fine, animals have to be sorted in groups and a main criterion for separation has to be defined. Groups and animals are defined as properties of the tracking area.
Settings have to be implemented in the *Animals and Groups* window (figure 18). This pop-up window appears when the *Animals* column in the *Tracking areas* table is mouse clicked (figure 17).

Into the *Group configuration* table you may enter a group name and you have to specify the number of animals in the group.

Animals of the same color

Size and *Marker* are criteria used for animals in the same group. Color is a criterion used for animals in different groups.
Size can be a good compromise for similar colored animals. It is always used along with *Likely movement*. And it is necessary that there is really a size difference. About 30 percent should be quite reliable. If the pixel masses are almost of the same size, it is not the best criterion. Because the size of an animal's pixel mass also changes when it rears or when it is grooming.

Putting a mark on an animal's back is certainly the most reliable method for preserving IDs. *Viewer* can check and correct the animals' identities with this criterion. In the current Viewer version this algorithm works with two animals in one group.

Marking can be done either by shaving a little whole that reveals skin – presumed it's not of the fur's color – or by painting the fur. To use this feature the default setting in the *Distinguish animals by* column in the *Animals and Groups* window (figure 18) has to be changed from *Likely movement* to *Mark.*

Animals of different color

Animals of different colors can be distinguished by the software based on their different contrast to the background. Configure one group for each color.

Examples:
- A black and a gray mouse: Two groups with one animal in each group.
- Two black mice and one white: One group for the two black and one group for the white animal.

Note: The best method to track differently colored animals is the color tracking mode. Although this method needs a color camera and thus can not be used in darkness with infrared illumination.

Tracking mode

There are three different ways the software can identify the animal in the video. When using a gray scale camera either use

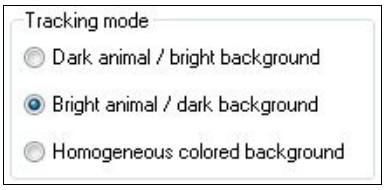


figure 19

1. *dark animal* on *bright background* or
2. *bright animal* on *dark background*.

Estimate the contrast ratio of your experimental setup by view. Accordingly determine the radio buttons in the *Tracking mode* field.
When using a color camera, you may use the third detection algorithm

3. *Homogeneous colored background.*

The essential difference between color tracking and contrast tracking is that objects of different colors and different contrast values can be identified. All objects in the area that do not fit into the defined color range, can be regarded as animal. As long as no animal is of the color of the background, it doesn't matters whether they look alike or not. In figure 20 an example is given where a black mouse and a red flower pot are simultaneously detected during filter calibration.

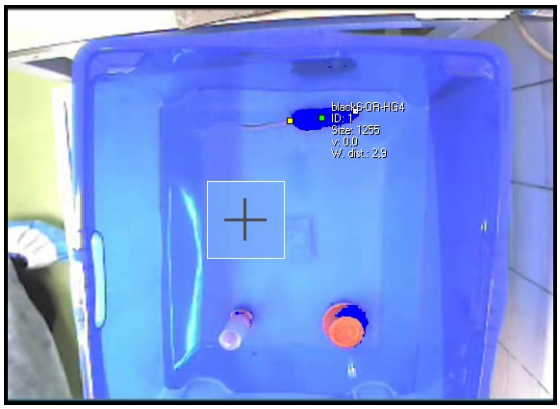


figure 20

All tracking modes base on the same idea. Identification of the background and subtraction of the animal. The following list approximately describes the sequence of operations.

__Get frame
____Identify pixel accumulations that do not belong to the background
_____Apply animal recognition filters to the pixel accumulations
_____Decide which shape pixel mass is the animal
_____fix head, body and tail position
_____Draw track to the animals last known position
__Get frame

Background filter

Actually digital videos consist of single pictures, comparable to celluloid movies. Filters in the tracking software examine each picture (frame) to distinguish between the animal and the background. Tracking is good when the object of interest is tracked permanently and exclusively. Therefore, filters have to be adjusted to work well in your specific setup.

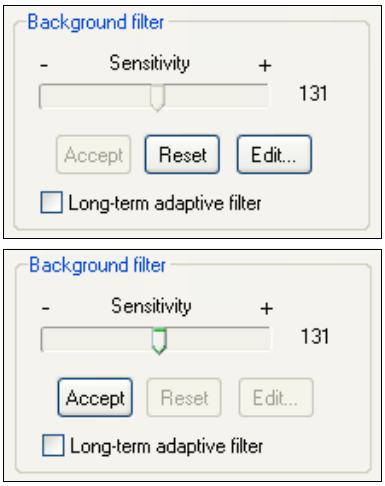


figure 21

Contrast tracking

The background filter is well adjusted if there is nothing but the animal in the video picture that the software tends to detect. Adjusting the background filter is done with the slide ruler in the *Background filter* field (figure 21).

a) To enable the ruler click the *Reset* button.
b) Move the ruler to the right or to the left to approach the threshold where the animal is blue (detected) but the background is not.
c) Remove the animal from the cage
d) Confirm the settings with the *Accept* button.

Immediately all pixels that possibly are points of an animal body turn from blue to red (figure 22 b).
By operating the *Accept* button the background filter is defined as brightness value.

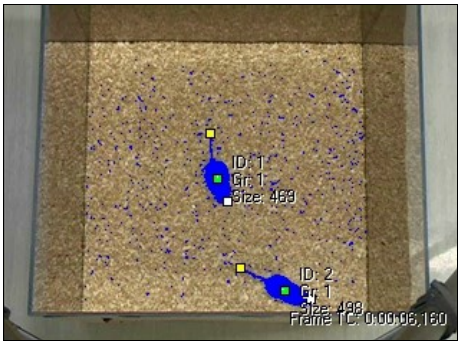


figure 22 a

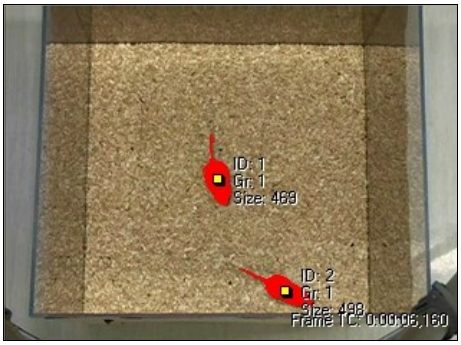


figure 22 b

If there where still animals in the cage when accepting the filter settings, the animals' pixels were detected at that very moment and thus painted into the background reference image. These pixels are now excluded from tracking because they are regarded as parts of the background that interfere with animal recognition (like in figure 23). You have to erase this snapshot from the background image. Otherwise the software will loose the track in these places.

e) Use the *Edit...* button (figure 21 a) to open a window that displays the currently set filter mask. On the left you find pencils and erasers to edit the mask.
The tool tip size is regulated by the value in the *size* field. Choose an eraser (white square or circle tool tip) to remove the excluded pixels from the mask.

If you adjust your filter settings and then remove the animal from the arena before you *Accept* the settings, reference image editing will not be necessary.

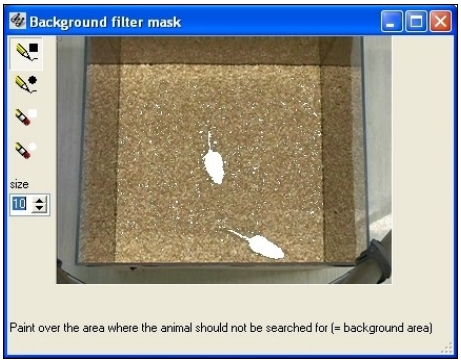


figure 23

With the pencil tools certain locations in the arena can be excluded from tracking at will. There might be an experimental construction in the arena that confuses the filter. For example a black object in an object recognition test with a dark animal on a bright background. Such an object can be covered with a spot to make sure, that it is not detected as animal.
After closing the window all background settings are adapted automatically. This procedure will help to maintain an optimal contrast recognition according to the contrast-filter-method, resulting in an optimal data recording. If you would like to change the filter settings, you can do that by clicking the *Reset* button again.

Note: By changing the background filter threshold, a new background image is established. Every time you reset the background filter threshold slide ruler, you should check the background filter mask image.

Color tracking

If you chose the third radio button in the *Tracking mode* box (figure 19), two more slide rulers and a button appear in the *Background filter* box.

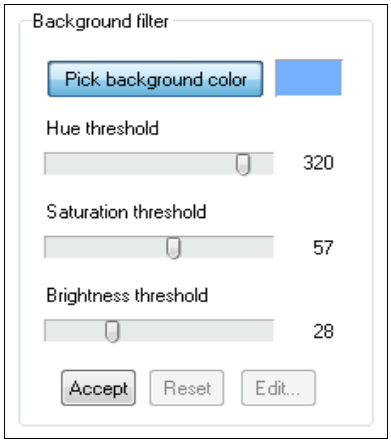


figure 24

a) Start adjusting by picking the background filter color. Therefore operate the similar named button (see figure 24) and move the mouse pointer over the video. The pointer turns into a plus shaped cross, surrounded by a magnified region of the image under it (see figure 20). To pick the color click on a spot (left mouse button click) where you think the color es representative for the whole setup. The picked color is now displayed in a field on the right side from the *Pick background color* button.

b) Then put the slide rulers to these positions to fill the complete area with blue color:

- *Hue threshold*: zero
- *Saturation threshold*: 100
- *Brightness threshold*: 100

figure 25 shows how to read the scales of these rulers. Hue is the color spectrum beginning and ending with nuances of intensive red. Saturation and brightness reaches from zero to 100, whereat zero percent color saturation and 100 % brightness value both lead to white as result.

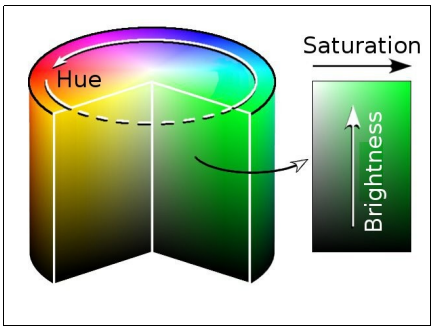


figure 25: picture from Wikimedia, edited

c) Move the *Hue* slider to the right, until no parts of the colored background are blue anymore

d) Move the *Saturation* slider to the left until some parts of the background become blue again. Then go back to the right just a little bit, until the background is clear again.

e) Finally move the *Brightness* slider to the left, until as much of the animal's pixel mass is blue but no background parts are included.

f) *Accept* the settings and edit the reference image as described in the paragraphs about *Contrast tracking*.

Long-term adaptive filter

The *Long-term adaptive filter* (see check box in figure 21) enables the *Viewer* to cope with the problem, that a test animal some times manipulates its environment during the experiment. Figure 26 shows a single rat in an object recognition test with two jars. The experimental setup is difficult to apprehend for the detection algorithm, because the jars – like the animal itself – are very much brighter than the background. So it is important only to wipe out the animal's silhouette from the background image and to leave the gaps in the *Background filter mask* that cover the jars. Otherwise three potential animal positions would be detected.

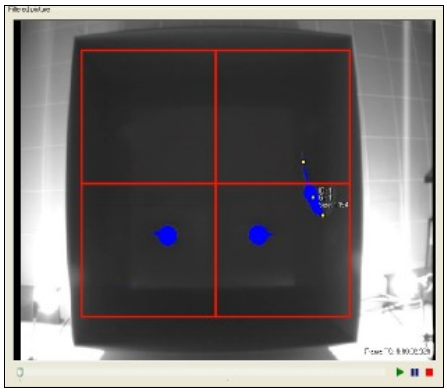


figure 26

If the rat relocates an object, it will not be covered by the gap in the *Background filter mask* anymore. The experiment could not be pursued.
But the *Long-term adaptive filter* causes the *Viewer* to capture new background images for each quadrant, where the animal is not present. This happens every time the animal changes from one quadrant of the arena to another. Relocated items will be detected and cut out from the new background image.

The result thus will suffer only from minor inaccuracies and the experiment doesn't has to be aborted. This is especially important, when long-term observations have to be made. To be honest: an object recognition test like in figure 26 is not the best example for this filter, because it last only a few minutes.
Since the *Long-term adaptive filter* claims a good portion of the computer's processing power, it will influence its total performance (e.g. recording frame rate) and should not be used, if it isn't really necessary. The algorithm is antagonistic to the *Reflection filter* (figure 36) algorithm, which is therefore suspended by it.

Animal filter

Once the background filter mask is set, the *Animal filter is* defined automatically by the software. Detected animals will be highlighted red in the filtered video picture. You can now manipulate the standard setting of the *Animal filter* manually by the upper slide ruler in the *Animal filter* box (figure 27). If you are not satisfied with the automatic setting, move the ruler to make as much of the animal as possible appear red.
The more pixels there are that can be included, the better detection will be, even corners of the setup with critical lighting conditions. But be careful not to include pixels that don't belong to the animal's body.

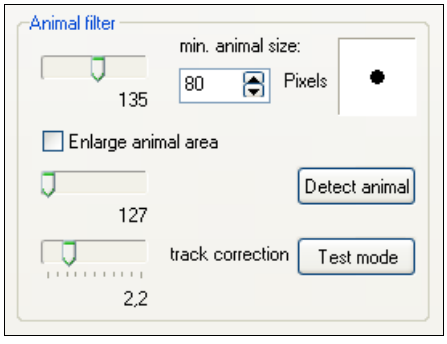


figure 27

In the *min. animal size* box you can enter a body size filter value. It determines a minimum size for the animal body in pixels. When the contrast filter detects objects smaller than the defined minimum animal size, they will not be tracked (for example feces, dropped during the experiment). That is also the reason why you have no red pixels in the background of figure Fehler: Referenz nicht gefunden b, although there were blue pixels in the background before the *Accept* button was operated (figure Fehler: Referenz nicht gefunden a).

To give you a feeling for the current minimum size, the entered amount of pixels is displayed as circle in the box next to the *min. animal size* field. In figure Fehler: Referenz nicht gefunden a and b you can see that the animal is denoted with its actual pixel size in the video picture, together with the animal *ID* and *Group*.

If you track animals of different colors (figure 28) or bi-colored animals in the same arena, you have to activate the *Enlarge animal area* function. This filter setting substitutes the combination of both tracking modes at the same time: *Dark animal/bright background* and

Bright animal/dark background.

Proceed as follows: First you have to choose one of the tracking modes. Select the one that refers to the animal that contrasts the background better than the other one. Then, adjust the filter's sensitivity (figure 21) in a way that the animal is just recognized, but not fully on target. Subsequently activate the *Enlarge animal area* check box (figure 27) and move the slide ruler until both animals are detected (figure 29). Finally, you can optimize detection strength by the animal filter ruler, but in most cases this should not be necessary. Comparing figure 28 and 30 evinces, that the three tracking points are well placed, even if recognition accuracy differs.

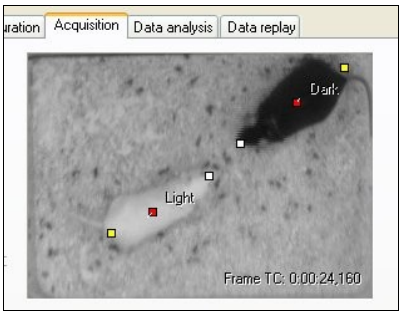


figure 28

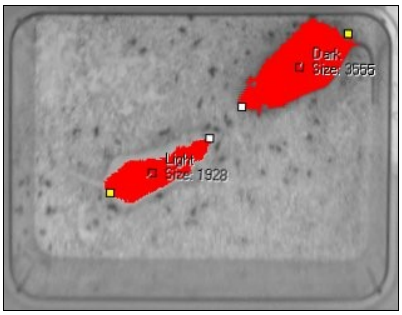


figure 29

The *Detect animal* button in the *Animal filter* box is a copy of the similar button under *Area info* box the on the *Experiment* tab (page 4).

Track correction concerns track length bias in setups with low lux values. Poor lighting leads to a grainy video signal. That is sometimes hard to see for the human eye, but it causes very fast track point tremble on a very small distance scale. This may sum up to remarkable track length measurement even when the animal is not moving. If you recognize this effect in your setup, you can encounter false measurement by using this tool.

Start *Test mode* with the similar named button to have the actual track length depicted near the animal (figure 30). Use an item as test animal dummy, so you can be sure there really is no action in the setup. Then move the slide control just until the depicted *Track length* value shows no development anymore (direction of movement depends on the *Tracking mode*). Don't move it to far beyond this point. Otherwise real movement track length could be pruned.

figure 30

The *track correction* value between zero and 20 is a number of pixels. If a movement from one frame to the next is lower or equal to the set value, the software assumes that it results from signal noise. In the result table, speed and distance will be set to zero for these coordinates.

Animal recognition settings

There are three X/Y coordinates per animal located in each video frame. These are the animal's center of gravity, the head and the tail end. The points are indicated by small colored squares. The squares' colors are free definable. Just click on a square in the color key in the *Animal recognition settings* box to open a color selection pop-up window.
Tail detection algorithms establish a posterior-anterior body axes alignment. In the *Viewer* there are two different algorithms for tail detection built in. With the default settings the *Viewer* examines locomotion direction and contour features. This strategy works in almost any case and has the advantage, that different lighting conditions in the setup have little impact on recognition quality.

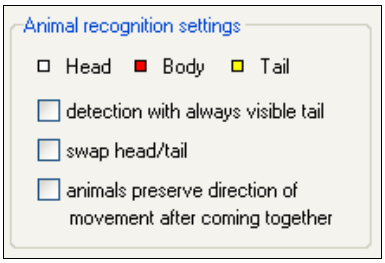


figure 31

Ont the one hand it delivers fine results as long as a tiny stub of the tail is detected. On the other hand it can not figure out if the contour includes a 'non-animal-region', like the two shapes on the right in figure 32.
The *detection with always visible tail* algorithm works completely different. It looks for tail-shaped silhouettes in the animal filter mask and allocates detected tails to detected animals. It can be applied alternatively (not additional) to the default algorithm. This setting makes sense, only if the animal's tail is always fully detected. Thus it can be used for rats, but almost never for mice.
In figure 32 detection was performed with *detection with always visible tail* algorithm. Without it, the sketch of the animal in the middle would have caused detection problems, due to its circular contour. Generally the default setting without accurate tail detection works best. Activating the check box for *detection with always visible tail* is recommended only, if the animals are good detected and quite passive.

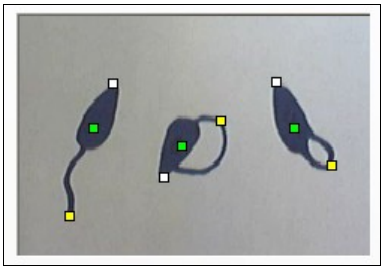


figure 32

If the tail is not detected at all, orientation recognition can be incorrect. Then you may have to swap head and tail reference points manually by activating the *swap head/tail* check box. It is useful to perform some test experiments to see how head/tail recognition works on your animals during locomotion. If your experimental setup and lighting conditions are very good and a tail is detected very well, you will not need to swap the points.

Maintaining the IDs of animals becomes more and more difficult, the more animals take part in a test. In 3D environments (aquaria) this is even catchier. Due to the two dimensional perspective of the camera, animals often appear as having contact although only their locomotion directions cross. The undermost check box in figure 31 (*animals preserve direction of movement after coming together*) switches on an algorithm, that is applicable for such a setup. In figure 33 the left example of track interpretation with two tadpoles would be enforced by the algorithm.

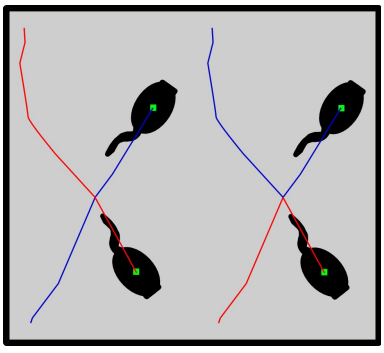


figure 33

Display settings

If you like to concentrate your own visual observation on the body point only, activate the check box *hide head and tail points*. Their coordinates will be tracked anyhow.

figure 34

Filters test run

Please check your settings by performing several test runs before acquiring data from real experiments.
On the *Acquisition* page you can switch on visibility for the current background filter mask and the long term filter. Select the similar named options from the context menu you can call with a right mouse button click on the video on the *Acquisition* tab. Figure 35 shows an example where a bright light reflection in the middle of the arena is covered by a spot in the filter mask. The spot in the filter mask is necessary because otherwise the huge reflection would be regarded as animal. On the other hand it prevents animal detection int the center of the arena.

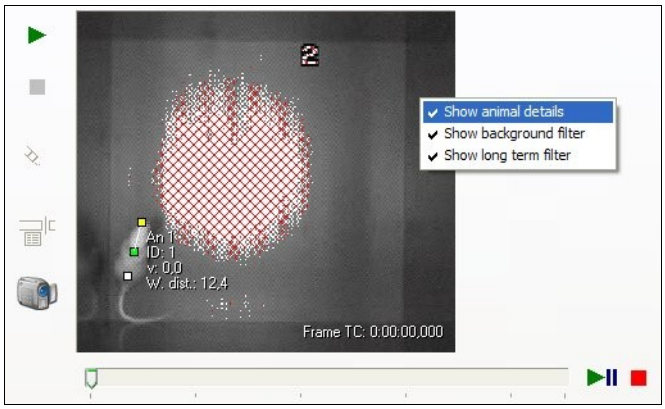


figure 35

When you watch a video or live experiment on the *Filters and Objects* tab, the detected animal is always indicated by a colored layer. The animal color is usually red. Sometimes it switches to other colors, indicating detected behaviors.

Animal and behavior detection:

red:	regular	detection
green:	headbob	detected
yellow:	headstretch	detected
magenta:	tailmove	detected
light green:	freezing	detected

(see also the table on page 32)

On the Acquisition tab, detected reflections are indicated by a green grid on white color (figure 38).

Reflection filter

The *detect reflection* function disbars artificial track points from being recorded as animal movement. These points are generated by objects that fit all filter settings but appear unexpected. For example points in the animal's mirror image in a perspex wall (figure 37) or reflections from waves in a water maze.
If such an object is detected, the track can jump there. The connecting section of the track is drawn from one frame to the next, thereby producing a sudden rise of velocity. Because of that, velocity is the parameter the *Viewer* uses to detect this kind of false tracking.

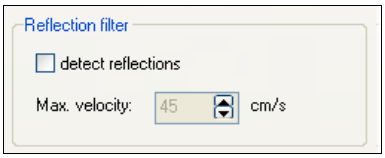


figure 36

When *detect light reflection* is activated, the *Max. velocity* input field becomes editable. Whenever the defined threshold value for this parameter is exceeded, a reflection is assumed and the target point is not rated as track point.

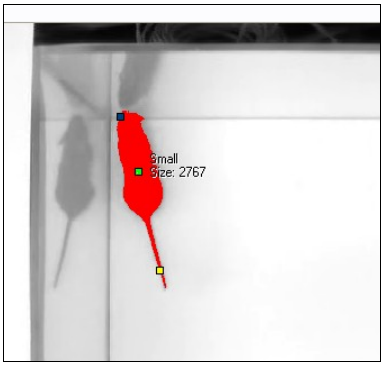


figure 37

What is a reasonable *Max. velocity* value?
The user has to identify the experimental animal's maximum velocity in pre-tests. The *Reflection filter – Max. velocity* should exceed this real value appreciably. Otherwise reflections might be measured in regular movement and correct track data is eliminated.
To prove correct settings, you can run an experiment and observe the video display on the *Acquisition* tab. Detected reflections are indicated as green checked pattern on white background.

Anyhow, if possible it should be avoided to be reliant on this kind of error correction. Building an adequate setup or drawing an observation arena that fits the floor without including the walls is the better solution (chapter *Tracking area*, page 9).

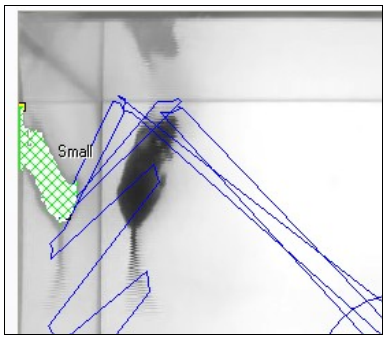


figure 38

Zone Definition

Drawing Zones – Modify a Zone – Duplicate a Zone – Delete a Zone – Zones Table – Reference Point

The software gathers data and calculates statistics for the whole arena and for each zone (track length, duration of stay, freezing, ambulation and more). During data acquisition area related data and zones' data are calculated and displayed in real time.
On the *Zone Definition* page you can draw zones to "harvest data from fields" in the arena. With zones it is possible to arrange a second, virtual setup in addition to the real experimental setup. Use zones to monitor behaviors and activity in locations the animal itself is not aware of as somehow different from the rest of its environment.

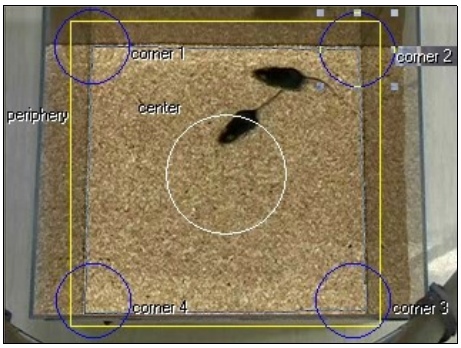


figure 39

Drawing zones

Like the areas, zones are defined by drawing them on the video. Selected zones can be edited. Selection from the table is done by clicking an arbitrary field in the zones table row (figure 43). Click on the zones rim with the the *Modify* cursor (arrow, figure 40) once to select a zone directly. Whenever a zone is selected, the border of the zone has square handles like the one in the upper right corner in figure 39.

Zones types

If you would like to use different individual shapes to define special areas of the arena, choose the *Rectangle*, *Ellipse* or *Free formed* button. Draw a zone into the video picture with the mouse while holding the left mouse button (*Rectangle* and *Ellipse*). Keeping the shift key on your keyboard pressed while drawing a zone, creates a perfect square, respectively a circle. When the *Free formed* tool is selected, surround the area you want to draw by clicking points on its margin. Lines will connect the single clicked points automatically. Set the last point near to the first one and the zone will be finished.
To add further zones, operate one of the three form buttons and mark the video picture again. To select a zone for further modification, switch to the modify cursor by the button with the arrow icon. That's the *Modify* button

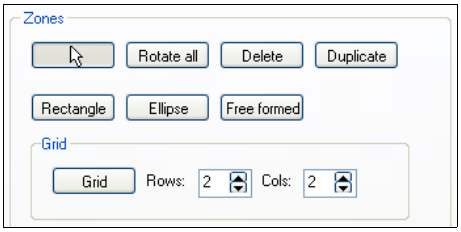


figure 40

Duplicate a zone

Zones can be duplicated by selecting the zone and pressing the *Duplicate* button. The new zone will be named *Copy of* ... This tool helps to provide similar zones in different arenas, if more than one area is tracked at the same time.

Delete a zone

If you want to delete a zone, select the zone and press the *Delete* button. Deleting several zones at once is possible, too. Use the *Modify* cursor to draw a rectangular selection. All zones that fit completely into the selection can now be deleted simultaneously.

Drawing a grid

instead of drawing individual zones you can draw a seamless grid of rectangular zones with the *Grid* tool. Click the *Grid* button (figure 40) and use the *Rows* and *Cols* fields to set the number of rows and columns in the grid. Now go forward as if you would draw a rectangular zone.

The rectangle you define will be subdivided into the number of fields you determined. Please note that the maximum number of rows and columns is limited to 10 each (100 zones).

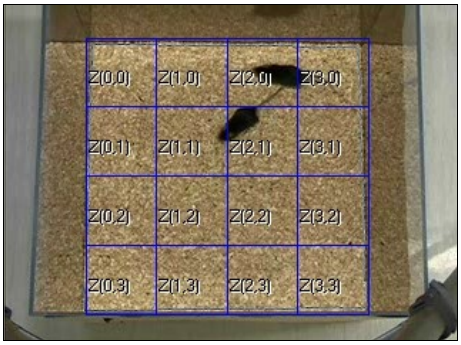


figure 41

Modifying size and position

Selected zones can be edited. Whenever a zone is selected, the border of the zone has square handles like the one in the upper right corner in figure 39. The squares can be dragged to transform the zone. Alternatively you can enter a selected zone's dimensions directly into the *Width* and *Height* fields into the table on the right side of the video display (figure 42). the unit in this table is pixels.

The yellow highlighted fields *Left* and *Top* can be used to determine a zone's position. In figure 42 the zero/zero position is indicated by the yellow dots on the video display.
You can move zones with the mouse pointer as well. To do so, select it with a single mouser click on the rim first (release the mouse button). Then click and hold the rim to drag the zone. Do not drag a square handle or the zone's label.

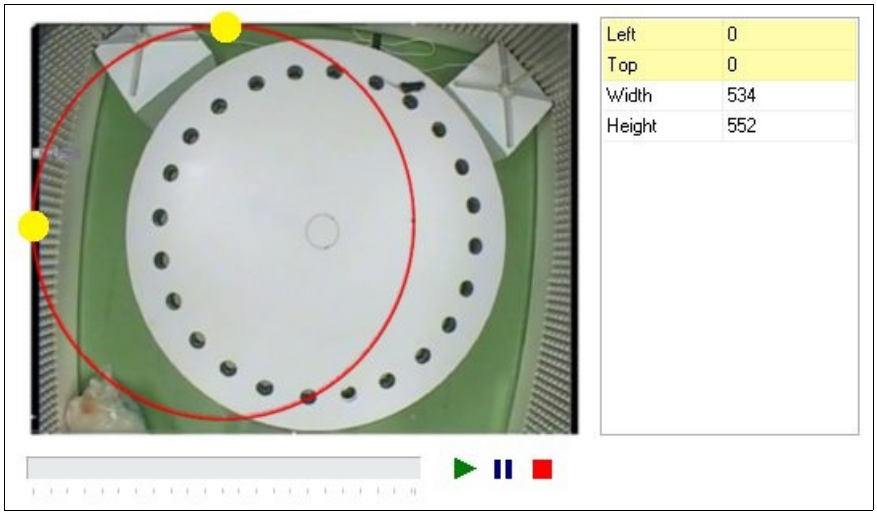


figure 42

At last the zone name can be moved. When the mouse is positioned over the name of an already selected zone it becomes a cross of arrows. You can now click and hold the label to drag the name to another place. Use this feature if zone names overlap or lie outside of the tracking area. During data acquisition the location of this label will be used to display zone data.

Zones table and zone attributes

The table in the *Zones* box contains information on existing zones and is a control panel for extra features. Each zone you draw is assigned with an ascending index number. In the table it is shown in the outer left column (*No.*). The number corresponds to the chronology of zone creation.
The *Show large view* button below the table opens the *Zones* table in a new window (figure 45), so you won't have to scroll to the right to see and edit all fields.

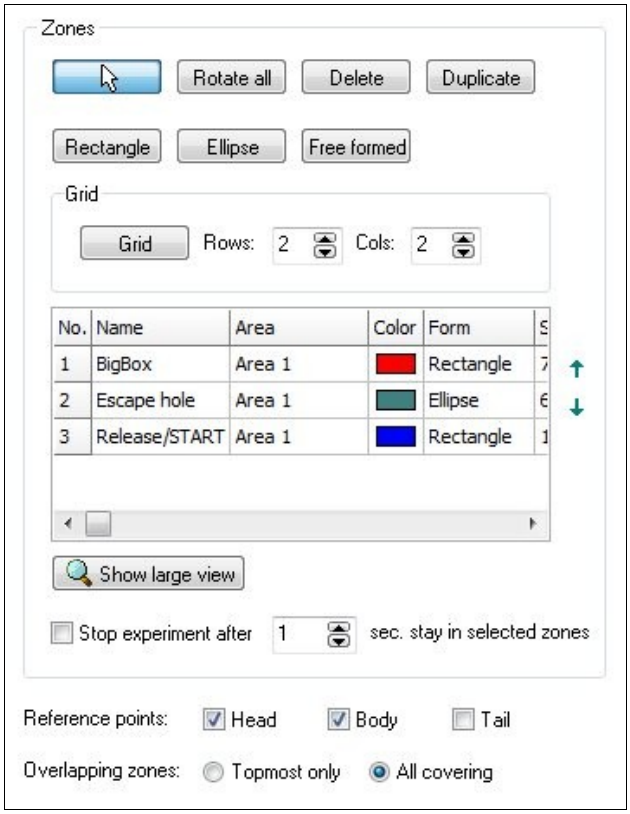


figure 43

Zone hierarchy

Defining a zone hierarchy becomes necessary when there are overlapping zones. The hierarchy then defines which zone is scored, when the animal is in two or more zones at the same time.
The way you want to handle overlapping sections is determined by the radio button choice *Overlapping zones* (figure 43 at the bottom).

Topmost only

Using this option means that the zones are handled like non-transparent layers (figure 44 a). The recorded data from the intersecting part will be assigned to the zone with the highest index number only (*No.*, left column in the table in figure 43).
Consider that the last table line from the top has the highest index number in the *Zones* table. This number is not fixed. It can be changed manually by moving a table line up or down with the arrow buttons on the table's right side. Select a zone first, then use the arrow buttons.
Example: You want to compare the time an animal spends in a marginal zone to the time in it stays in a center zone. Draw a zone that fits the size of the arena first. Then draw a second zone in the center of the area. Whenever the animal is in the center zone, track data will exclusively count in the center zone due to its higher rank (zone # 2 overrules zone # 1).
You can not edit the index number directly, but you can change each zones position in the table and thus its ranking by using the turquoise arrows on the right side of the table.

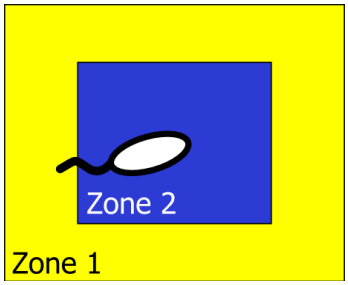


figure 44 a: Topmost only

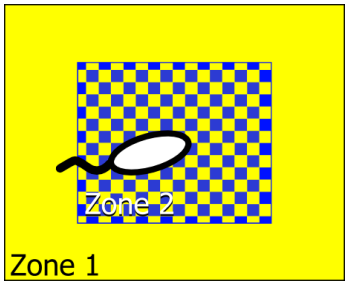


figure 44 b: All covering

All covering

With this option there is no hierarchy at all. All zones always get scores and as consequence the sum of all zone data can be more than the area data if zones overlap. For example more track length.

Experiment | Camera | Filters and Objects | Zone Definition | Activity | Behavior | Saving Options

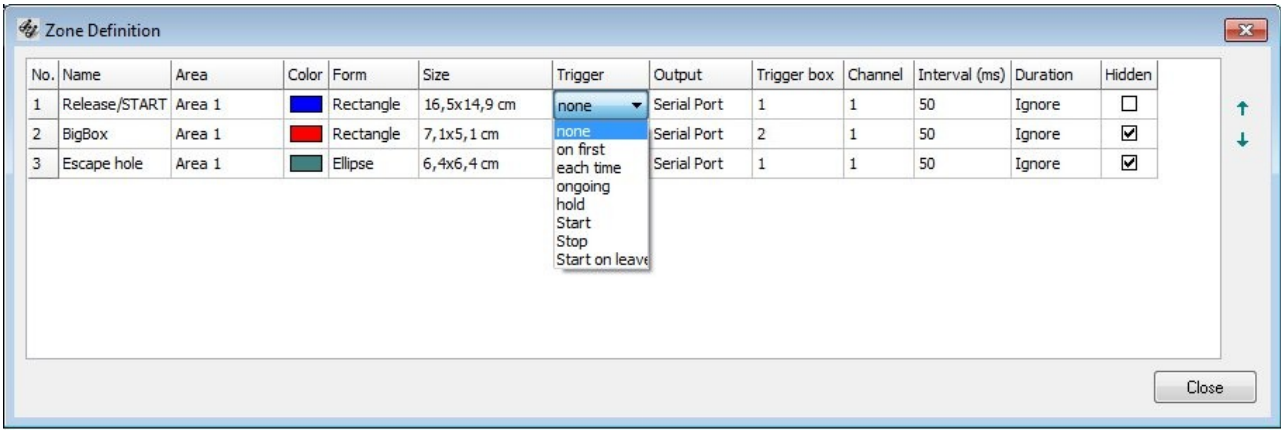

Zone Definition
No. Name Area Color Form Size Trigger Output Trigger box Channel Interval (ms) Duration Hidden
1 Release/START Area 1 Rectangle 16,5x14,9 cm none Serial Port 1 1 50 Ignore
2 BigBox Area 1 Rectangle 7,1x5,1 cm Serial Port 2 1 50 Ignore
3 Escape hole Area 1 Ellipse 6,4x6,4 cm Serial Port 1 1 50 Ignore
none
on first
each time
ongoing
hold
Start
Stop
Start on leave
Close

figure 45

Zone name

Newly drawn zones will automatically be added in the zones table. Their default names are Zone 1, Zone 2, etc. You can type your own zone names into the fields in the *Name* column.

Zone to area affiliation

It is ensured that data from simultaneously observed areas is not mixed up because zones belong to one area only. That is the one area where the zone's center point lies in. A zone's 'membership' is depicted in the *Area* column in the zones table. If you move a zone so that its center point comes to lie outside any area, its field in the *Area* column will be empty and no data will be collected fort that zone.

Zone color, shape and size

Changing a zone's color (click the field in the *Color* column) affects the contour color and the color of bar and line charts that refer to the zone statistics. The shape of a zone is noted in the *Form* column, the expanse in the *Size* column.

Trigger-zones

Zones can be used to trigger external devices or acquisition start and stop. While the already bespoken *General Trigger control* features (*Configuration – Experiment* tab) are time dependent, the *Zones* table offers advanced options for zone visit dependent feedback.

a) First select a trigger mode from the drop-down menu in the *Trigger* table field of a zone in the *Zones* table (figure 45). There are two different types of triggers in this menu:

Signal output control

- *none*: default setting; no action or trigger signal
- *on first*: carry out the action only the first time the animal enters the zone
- *each time*: carry out the action each time the animal enters the zone
- *ongoing*: repeat the trigger for the whole time the animal is in the zone (by use of the set interval)
- *hold:* send a permanent TTL pulse as long as the animal stays in the zone (no interval)

Data acquisition control

The last three options in the menu are meant to control *Viewer* data acquisition itself:

- *Start*: Start the experiment as soon as the animal enters the zone
- *Stop*: Stop the experiment as soon as the animal enters the zone
- *Start on leave*: Start the experiment when the animal leaves the zone

Acquisition *Start* and *Start on leave* work regardless the zone's hierarchy. That means that even if you decided to work with the *Topmost only* hierarchy (see *Zone hierarchy* paragraph, page 22), you can use a zone that is covered by another one to start the experiment.

Note: *Start on leave* zones are special concerning data acquisition. Data like visits, track length and all the other parameters are ascribed to a *Start on leave* zone only in *Topmost only* tracking mode. In *All covering* mode the zone is treated like any other.

This offers the possibility to use the start zone functionality without producing statistics.

b) As soon as a *Trigger* option is defined, the other necessary fields in the table line become editable. In the next step one of three output options has to be defined:
Either *Serial port* or *Parallel port* can be chosen from the drop-down menu in the *Output* column.
If you have a BIOBSERVE *Trigger box* connected and a COM port for it is chosen under *General trigger control*, you can select the box from the *Trigger box* table field. Select box 1 or 2 from the drop-down.

c) Each *Trigger box* has eight output channels. Select the Channel you want to associate with the zone from the *Channel* table field of the zone.

d) At last you may define an interval for the *ongoing* trigger. Simply enter a value in milliseconds into this field.

Note: Single pulse duration is always 20 millisecond.

Summed up time as stop trigger

There is another way to stop acquisition in subjection to zone data. This time, duration of stay in different zones is summed up. First tick the check box

Stop experiment after [ ] *seconds of stay in zones*

under the *Zones* table and enter a value into the empty field. Subsequently all zones with the *Summarize* attribute in the *Duration* field in the *Zones* table will contribute to the time limit you entered.
Of course, you may use different stop functionalities at the same time.

figure 46

figure 47

Hidden zones

For each zone there is a check box in the outer right column, to switch on or off the *Hidden* attribute. Such a zone is used to locate a hiding-place. For example when the test animal sits in a provided box. Whenever the animal is not detectable for the computer, the system assumes it is in the center of the *Hidden* zone. So you get a period of constant location information.
Always the *Hidden* zone next to the last detected moving center point is used as assumed location, when more than one zone carries this attribute.

figure 48

Reference point

Some technical terms have to be discussed for a complete understanding of the meaning of the *Reference point*. First, there are three track points placed on the animal: *Head*, *Body* and *Tail*. Each produces an X/Y coordinate data point in each single video frame. The visualization of these points is the track. Each track point produces its own track. You can visualize these tracks during data acquisition (see page 36).
To define a single *Reference point* activate one of the check boxes in the *Reference point* field (figure 49).

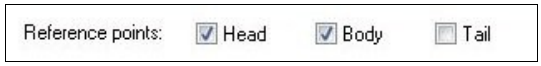


figure 49

To keep in mind:

1) **Zone visit related parameters refer to the *Reference point(s)*.** (duration of stay in a zone, zone visit count, visit latency)
2) **Locomotion related parameters refer to the animal's *Body* point only** (distance, velocity, activity)**!**
3) **Trigger rules refer to zone visits** and thus to the *Reference point's* position as well.

The *Reference point* is merely decisive for the zone entry and exit but does not determines the section of the body track, that is scored as belonging to a zone visit.
The first sketch in figure 50 shows a section of the *Body* track that lies completely inside the zone. That is how *Body* track parameters are scored for the zones, regardless the *Reference point* settings.
The other three sketches show, that zone entry and exit are logged a little bit delayed to the *Body* point's entry when *Head* or *Tail* is selected as *Reference point*.

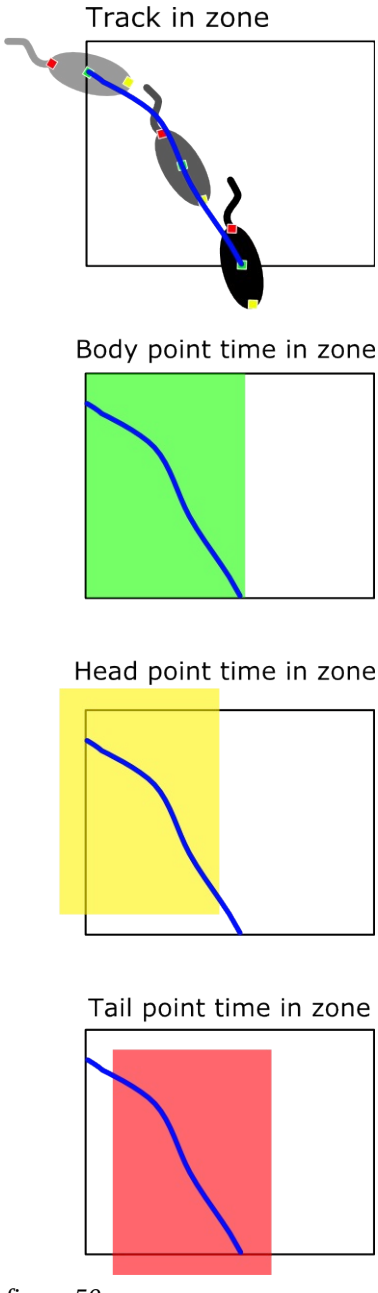
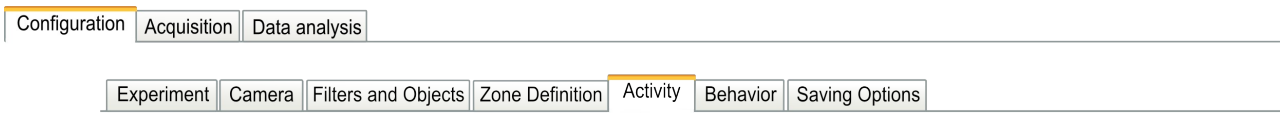


figure 50

Multiple reference points

It is also possible to define more than one *Reference point*, as you can see in figure 49, where the *Head* and the *Body* check box is activated. The difference is that both or all three *Reference points* must enter the zone or mus exit the zone, before a visit is scored or ended. This feature is suitable to prevent unwanted repeated zone visit counting. That can happen with one *Reference point* when an animal moves along the edge of a zone or sits on the edge and just moves a little bit back and forth.
Use two or three *Reference points* if you are mainly interested in the visit counts, like in plus or Y maze!

Example water maze:
In a water maze test you might want to prevent that a single swim across the target platform is immediately scored as success. Instead of defining a stop trigger for the platform zone you could make the *Viewer* stop acquisition when the whole animal rested on the platform for three seconds.
In that case, determine the *Tail* and *Head* (or all three) points as *Reference points*, mark the *Summarize* attribute for the platform zone and insert '3' into the *Stop experiment after* __ field.



Activity

Velocity Class Definition – Activity Threshold – Wall Zone – Zone Crossing

On the *Activity* page different parameters to define and measure activity in your tests can be configured. Configuration of these parameters is not obligatory.

Velocity classes

In the *Velocity class* definition table you can define up to 10 different velocity classes. The time an animal spent in these classes will be automatically quantified during data acquisition. The lower reference value of the first velocity class is fixed to 0,0 pixels (cm, mm, inch, if calibrated) to record immobility. You can determine an upper limit by clicking once in the appropriate box in the *To* column and typing in the desired value. After confirming your choice by the enter key, the limit will automatically appear in the next velocity class as the lower limit. Tip: use this method to finish defining the remaining undefined velocity classes. In order to have a useful division of classes that is well-suited to your experiment, you have to know the speed the animal can reach.
In contrast to the fixed lowest class the highest class is free definable. You might define very narrow classes to investigate on small differences in a certain velocity range or split uniformly from the lowest to the highest value.

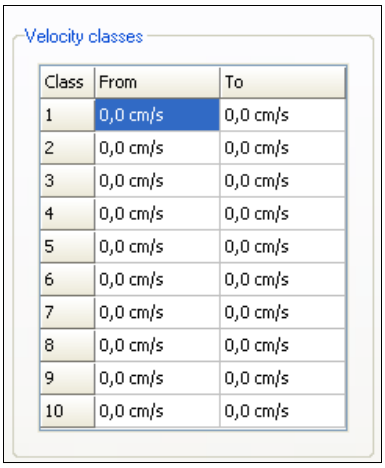


figure 51

Activity threshold

The *Activity* threshold is a velocity limit to distinguish active from inactive behavior. It is a track related parameter that is calculated for the whole arena and zone based. So you can, for example, enter (manually or by arrow keys) that the animal applies to be active, if it moves at least 0,10 cm per second. The issued *Activity* parameter is expressed in percent of total experiment time.

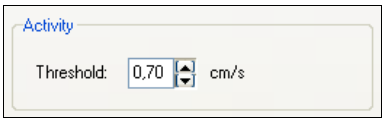


figure 52

Wall zones

The *wall zone* parameter informs about the animal's body point distance to the nearest wall at any time of the experiment. It is often used to draw a conclusion about the animal's anxiety. In an open field experiment, for example, it delivers much more convincing data than the common center zone visits.
To measure the distance, the arena's walls have to be defined. In many cases the tracking area matches the walls of a setup quite good, but in some cases not at all. Figure 53 shows a rectangular setup that is covered with a rectangular tracking area. But the camera distorts the image a little bit, so the *Wall zones*, which are outlined by the blue lines, are better drawn with the *Free formed* tool. *Wall zone* drawing is done in the same way like creating a tracking area. It is possible to define one *Wall zone* per tracking area. The tools are placed on the left side of the video image on the *Activity* tab.
With the *Delete all* button you can remove all existing wall zones at once. The tool is especially helpful, when you cannot find a zone you want to delete in the video frame. For example due to a clipping caused by changed calibration.

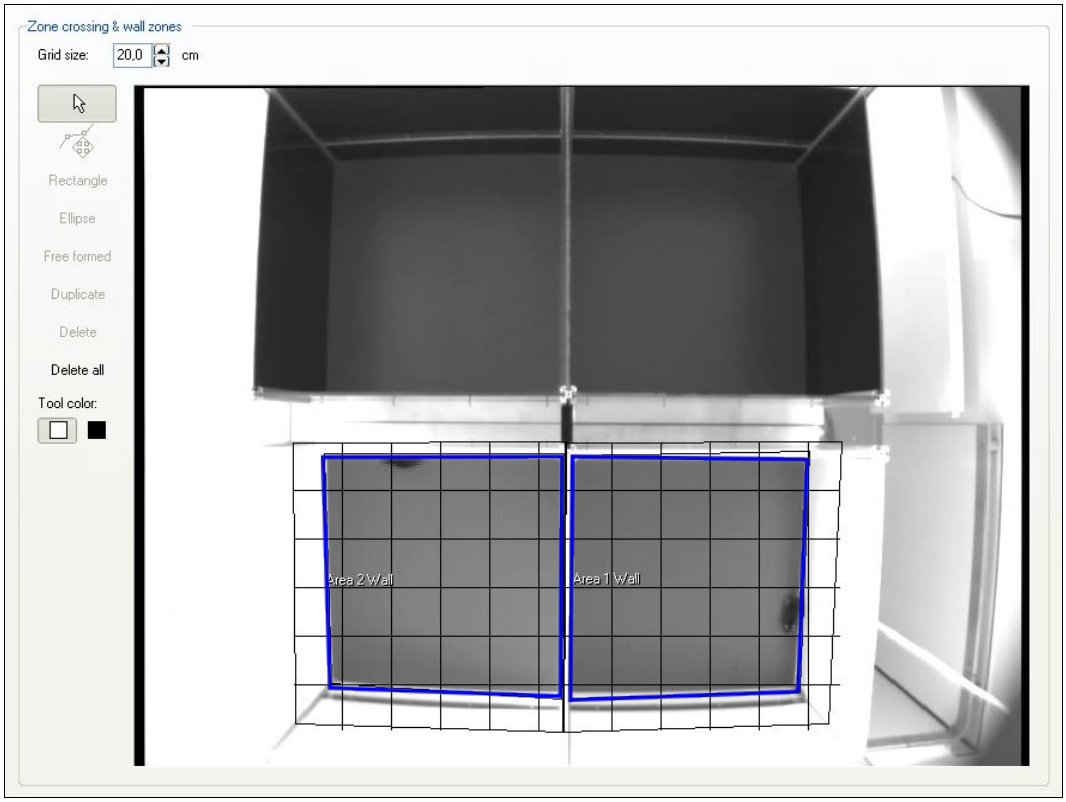


figure 53

Area covered

Area covered is an activity parameter. In the result it is listed under *Area data*. It has its own charts on the *Acquisition* page and on the *Data analysis* page.
Scores for the *Area covered* parameter are issued as percentage value. Meant is the percentage of visited area inside the *Wall zone* during an experiment. If there is no *Wall zone* defined, there will be no results for this parameter.
The smallest visited area unit is defined as one grid cells from the *Zone crossing* grid. So there is no explicit threshold to adjust, but three variables have to be thought of.

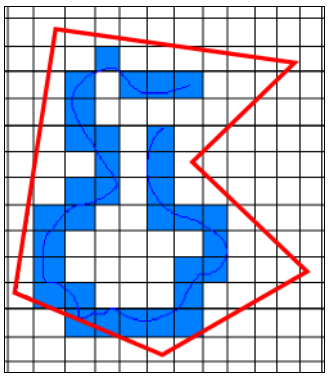


figure 54

The variables for parameter calculation are:
- the body track point as criterion for grid cell visits,
- the *Zone crossing* grid size that determines the size of the cells, and
- the *Wall zone* to determine the reference pane.

As a rule of thump it is recommended to choose a grid cell size that maps at least the animal's body size. Smaller cells lead to very low *Area covered* values.

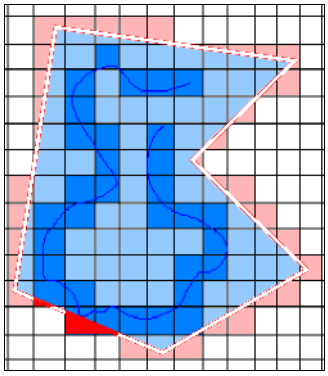


figure 54 b

In figure 54 the red polygon is the *Wall zone*. The dark blue line is the track. The lighter blue squares are visited *Zone crossing* grid cells. Each cell is counted once, regardless how often it was visited.
For the polygon the exact surface area is calculated in square pixels. The area of a cell is also defined in square pixels and the index is then calculated by use of this formula:

$$\left(\frac{\text{Number of visited cells X cell area}}{\text{Wall zone area}}\right) \text{X } 100 = \text{Area covered}$$

Note: The *Covered area* result is a nice and expressive value to compare animals' activities in the same setup. But you have to be careful comparing results from different setups. Not only the grid cell size has a high influence on gained data. Also the outer shape of the *Wall zone* is crucial. In the free formed zone example in figure 54 b you can already see, that it is possible to achieve values higher than 100% by visiting cells that are cut off by the *Wall zone* (light and dark (visited) red cells). These cells count with there full area. You can minimize this effect by using smaller grid cells. But small cells are not that suitable to describe the actually explored area.

Actually you have to find and keep settings that deliver reasonable results in your eyes.

Zone crossing

The *Zone crossing* index evolved from a former method of manual measurement. Scientists used to draw or glue a grid of lines on the arenas floor and counted the zone crossing events to estimate the track length and thereby activity. The *Viewer* generates this parameter to offer comparability to older data.
In this context the terminology needs clarification: Although the grid lines form rectangular zones, actually the line crossing events are counted. There are no zones of the type we define on the *Zone Definition* tab.

The *Viewer* zone crossing measurement delivers two parameters: *Zone crossing* and *Rated zone crossing*

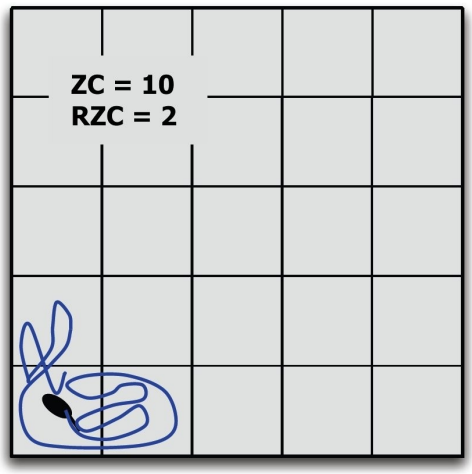


figure 55

Zone crossing (ZC)

Unrated zone crossing represents the effective number of line crossing events.
High *ZC* values can result from minimal but frequent position changes in the closest vicinity of a grid-line.

Rated zone crossing (RZC)

Rated zone crossing implements a rating that emphasizes linear movement and discounts small-scale locomotion. In *RZC* each line crossing event is weighted by a discrete factor between zero and four (see the formula below). The factor is built by looking at the last three line crossing events, whereat the distances from the first two to the last are summed up. The distances are expressed as number of necessary line crossings to get from the starting zone to the final zone. Finally, one count is subtracted. Thereby track oscillations at only one zone's margin are leveled to zero.

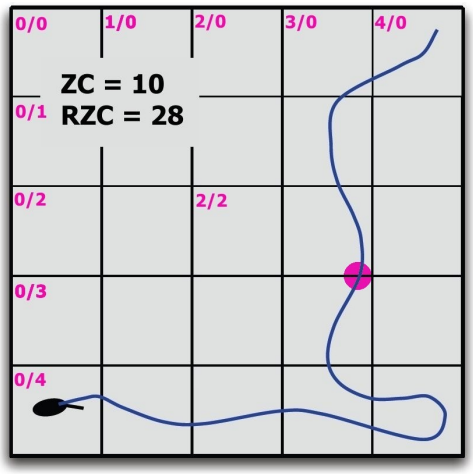


figure 56

Example: The dot-marked line crossing in figure 56 symbolizes a transition from cell 3/2 to cell 3/3.

It is rated as follows:
Reference starting cell is No. 3/0
(3/0 to 3/2) + (3/0 to 3/3) - 1 = 2 + 3 - 1 = 4

Rated zone crossing weight factor:

$$\sum_{n=1}^{n} \overline{(N_{(n+1)}, N_{(n+3)})} + \overline{(N_{(n+1)}, N_{(n+4)})} - 1$$

Behavior

Headbobs – Headstretches – Tailmoves – Freezings - Ambulations

Besides pure tracking, the software can differentiate and register some typical rodent behaviors – even in the *Viewer* standard version. On the *Behavior* tab detection algorithms for these behaviors can be adjusted. You can define recognition thresholds for the behaviors headbobs, headstretches, tailmoves and freezings. Activating the corresponding check box is precondition for automated behavior recognition, in each case.
The color code in the upper right corner of each behavior is used on the *Filters and Objects* page to indicate a recognized behavior (see page 18).

Headbobs

Headbobs indicate that the animal orients itself. If you don't want the software to record this typical orientation behavior when the animal simply bends its body axis walking around an obstacle, you can determine a moving velocity limit in the *Animal velocity limit* input field (upper threshold). The unit depends on your calibration (cm, mm, inch) or otherwise is pixel/s. Assumed you enter zero, headbobs will only be recognized if the animal's body point is absolutely fixed. As this is almost never given, an apposite minimum velocity should be assigned.
The further the *Head movement threshold* sliding control (lower limit in degree per second) is moved to the right, the faster the head movements have to be, in order to be recorded as headbobs. Control your settings based on the corresponding sketch. It shows a pixel mass that moves exactly at the current threshold speed.

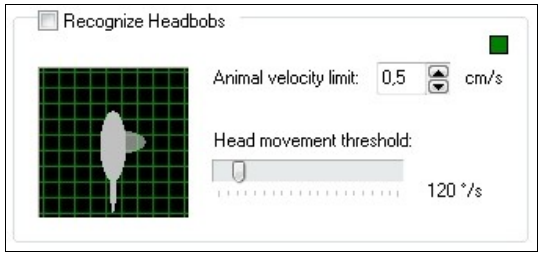


figure 57

Headstretches

Headstretches are used to differentiated sniffing and exploration behavior from simple forwards directed locomotion. Configuration of headstretches is nearly similar to the previously explained headbobs configuration.
The only difference is, that the sliding control threshold value for headstretches is adjusted in length per second, not in degree/s.

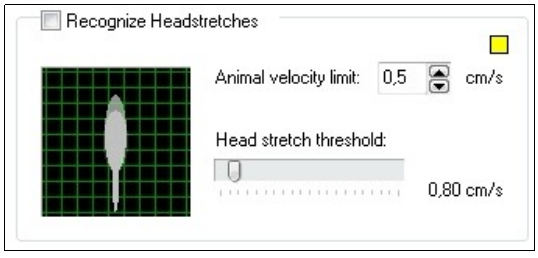


figure 58

Freezing

Mice may show freezing behavior when they are timid or startled. They stand still on a spot and don't move. To distinguish between freezing and short stops, freezing behavior is determined by the *Animal velocity limit* (length/s) and the *Minimal duration threshold*. The last threshold is a time in seconds the animal has to stay immobile, according to the *Animal velocity limit*.

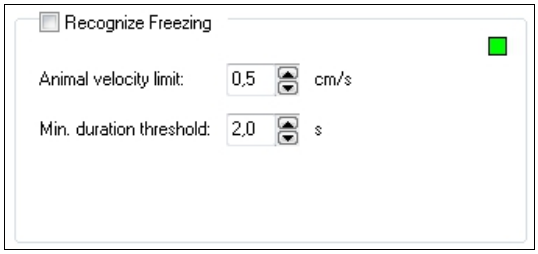


figure 59

Tailmoves

Tailmoves are likewise defined as *Animal velocity limit* (length/s) and *Tail movement threshold* (degree/s).

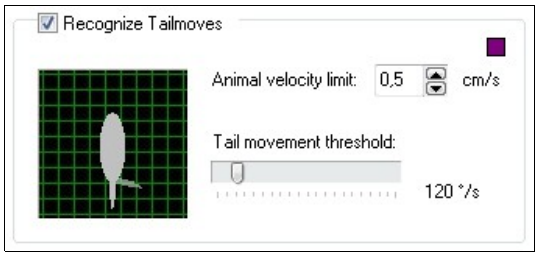


figure 60

Ambulations

Ambulation behavior is defined as a spontaneous short term acceleration. It is a function of the animal's velocity that requires a significant change within three video frames, as figure 62 shows it.
With the arrow keys you can lower or increase the velocity threshold for the registration of *Ambulation*. The also used *Activity threshold* is defined on the *Activity* page (page 26).
Statistics on *Ambulation* behavior are issued for the arena and the distinct zones as total count of recognized events. In *Zone data* the event is scored for the starting point of the ambulation track

figure 61

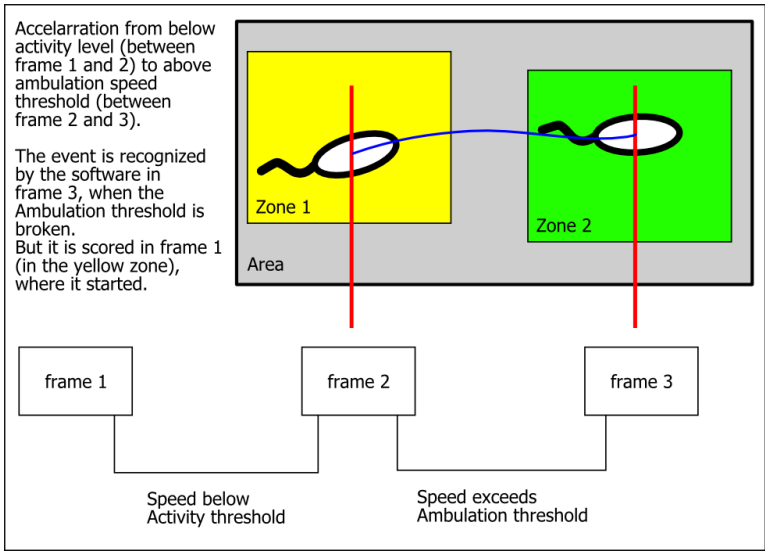

Accelarration from below activity level (between frame 1 and 2) to above ambulation speed threshold (between frame 2 and 3).
The event is recognized by the software in frame 3, when the Ambulation threshold is broken.
But it is scored in frame 1 (in the yellow zone), where it started.
Zone 1
Zone 2
Area
frame 1
frame 2
frame 3
Speed below Activity threshold
Speed exceeds Ambulation threshold

Figure 62: A measurement over three successive video frames

		Indicated by color on the *Filters and Objects* page
Headbobs	the animal moves its head horizontally; recognized by change of angle between the *Tail* point – *Body* point line and the *Body* point *Head* point line	dark green
Headstretches	the animal stretches its head; recognized by the change of length of the *Head* point – *Body* point line	yellow
Tailmoves	the animal moves its tail; recognized by change of angle between the *Head* point – *Body* point line and the *Body* point *Tail* point line	magenta
Freezing	the animal remains motionless for a while	light green
Ambulation	The animal accelerates very fast from inactivity beyond the set *Ambulation* threshold	

Saving Options

Summary – Movie Recording Setting

Summary

In the Summary field you can determine a time interval in which recorded results of running experiments are summed up (see results table on the *Acquisition* page). The summarized interval results will be consecutively presented during acquisition. Use a short interval, if you want to see details in parameter progression. Use a longer interval, if you want to evaluate events in time bins. For examples ambulations per minute.

figure 63

Movie Recording Setting

Record movie

If a camera is connected you can save a video file while the experiment is running. Therefore check the *Record movie* check box. The movie file (avi format) will be saved in the *Data* folder. Its file name is identical to the result file name in the same folder, so you can recover it simply by sorting by file name in your file browser (Windows Explorer).
To save a video file it is absolutely necessary that you save experiment results directly after acquisition, when the save dialog pops up. Only then the until now temporarily saved video is filed to the data folder permanently. If you cancel this dialog and save results later, the video file will not be included.

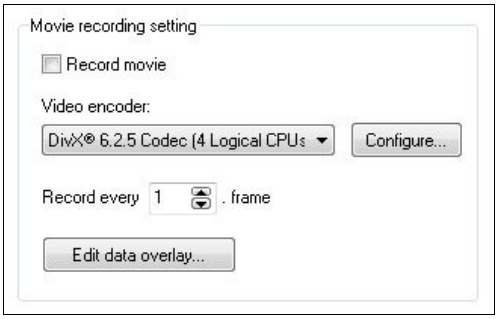


figure 64

Video compression

To encode the analog video signal to a digital movie file, the DivX Pro video codec must be installed on the computer. In case you are working with a system delivered by BIOBSERVE GmbH, the codec is already installed and properly set up.
Use the *Configure...* button to open the configuration window of the DivX encoder (external program).

It is possible to reduce the saved frames by the *Record every ___ . frame* input field. This can be reasonable if you are afraid that your hard drive capacity might not be enough for a long term recording.
For most tracking task 15 frames per second (fps) are enough data points for a reasonable analysis. With an NTSC camera that delivers 30 fps and only every second frame recorded you can spare 50 % storage capacity. But generally BIOBSERVE doesn't recommends to reduce the frame rate in the saved videos. Nowadays storage volumes are big enough for almost any purpose of saving.

Data Overlay

The *Edit data overlay...* button opens a window where you can add data fields from the *Viewer* directly into the video. This works when a live signal is recorded. It is not possible to edit already existing video files.
You can add for example experiment start and stop time, the animal's name and much more (figure 65). The feature can be used to identify experiments in the saved video.

Note: Be careful not to place theses data fields over the section of the video image you defined as tracking area. The data overlays do not take influence on live tracking, but a data field in the tracking area would make it impossible to reanalyze the recorded video properly.

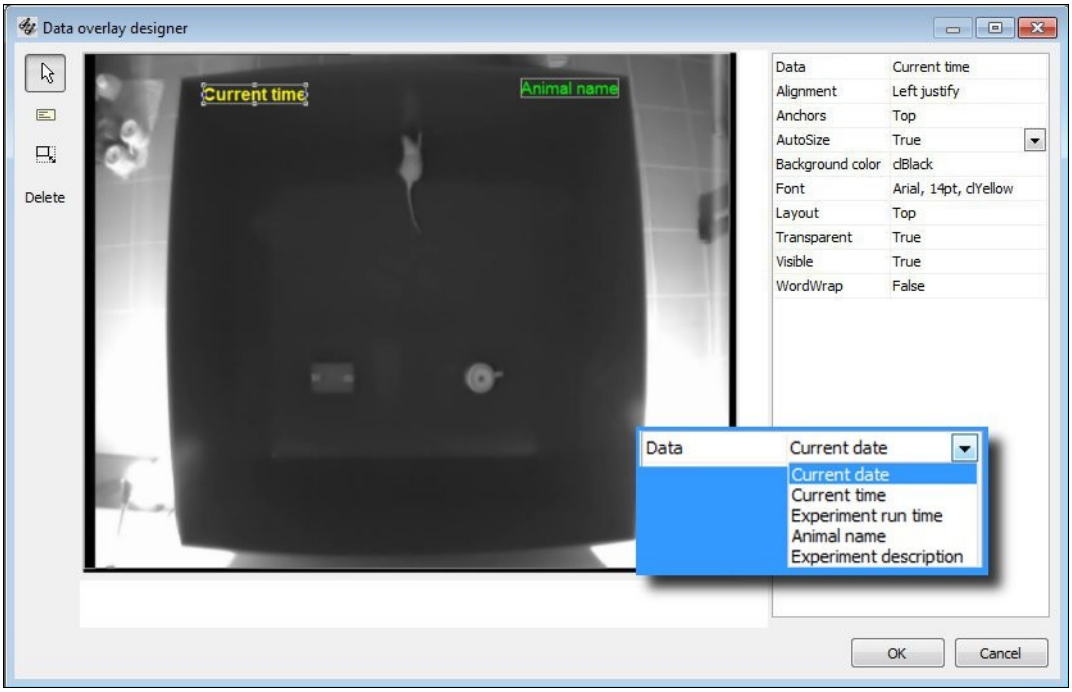


figure 65

On the left side of the video are the tools to create, to place and to delete data fields. The second tool from the top is the *Add field tool*. When this one is selected you can draw a rectangle somewhere on the video. After releasing the mouse button you can edit this field by changing the content in the input field on the right side.
The input fields are arranged in a table with labels in the left column and content drop down menus in the right column. In figure 65 the *Data* input field is shown twice: In its actual position in the table and additionally in the blue shaded box where it is a bit magnified and its attached menu is dropped down. There you can read the dates you can select. All other input fields concern display options.
The *Font* field and the *Background color* field have no drop down menu. A mouse click into these fields opens a pop-up dialog where new settings are implemented.

Saving dialog

Viewer can assist configuration saving to make sure that wanted changes don't get lost. For example an other animal name or zone settings.
When a configuration is unloaded *Viewer* prompts for configuration file saving. This happens whenever *Viewer* is closed, every time a new configuration is loaded and every time a result file is loaded. If you don't need this feature, switch it off by activating the upper check box in figure 66.

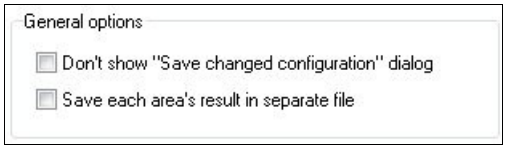


figure 66

With the *Save each area's result in separate file* option it is possible to produce distinct VRF files for simultaneously observed tracking areas. This setting is recommended. It provides exact experiment to file assignment and produces smaller files that are easier to handle in data bases or if reanalyzing becomes necessary.
File naming with this option is simply: user-given-file-name_area_number.vrf
For example: openfield_area_2

File paths

It is useful to define default paths for the MS Windows save and open dialogs for different file types to make file browsing easier. This is done in the *File paths* box (figure 67).
Operate the button with the three dots on the right from a path. Browse to the folder you want to determine as default location and confirm with *OK*.

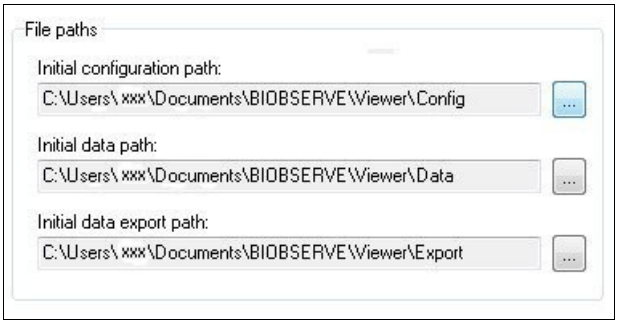


figure 67

In Windows 7 you need administrator rights to choose any other folder than sub-directories of ...user\Documents\..
or the *Viewer* program folder.

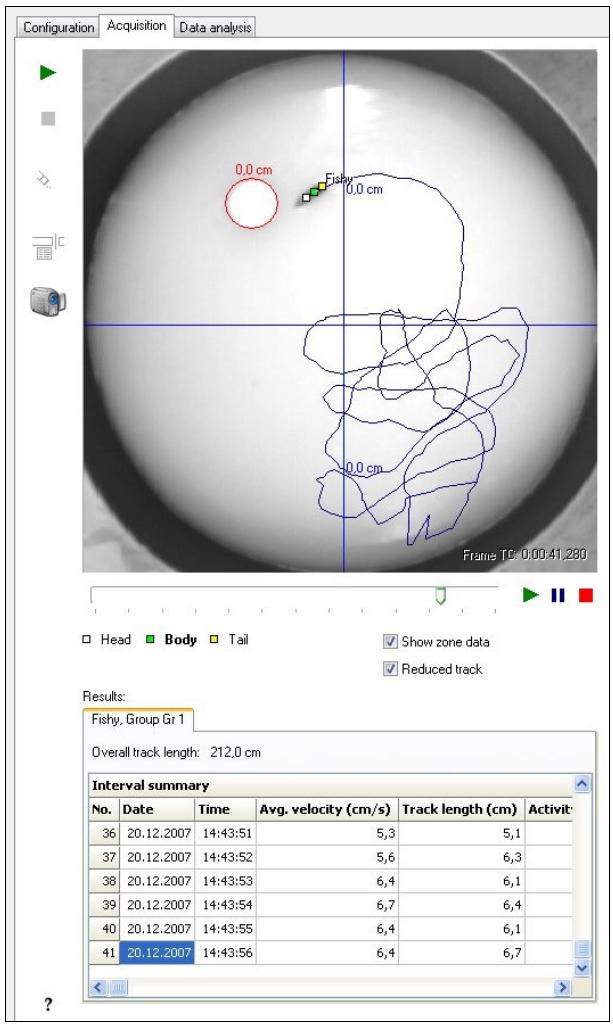
Video files are automatically saved in the same directory as result files. However, it is possible to load video files from other directories. *Viewer* remembers the last used location and opens the same folder again, once you browsed there and as long as *Viewer* is not closed.

Acquisition

Page architecture – Start and stop Data Acquisition – Video Display Options – Online Statistics – Raw Data

Page architecture

As soon as a video source is selected and a suitable configuration is loaded or created you can switch to data acquisition by clicking the *Acquisition* tab.
In the upper left corner of the window you can see the part of the video that is inside the defined tracking area. Track points are visible on the animal's body. In proximity to the body reference point of each animal the animal's name and group number (*Gr*, if existent) are indicated.
Below the video image a legend with colored boxes reminds of the track points' colors. You may change colors. Simply click on one of the squares to open a color customizing window.
By default the *Body* point track is visualized. This is indicated by the bold font of the label in the color key. If you want to visualize another track, simply click on the respective track point label.
In the lower left corner summed up interval data is shown in a table below the video picture during the experiment (figure 73).
The raw data table in the lower right corner displays tracking points coordinates during acquisition. The rest of the right window side is filled with graphs. Values for each parameter are visualized there in real time. Available features and graphs will be explained in detail in this chapter.
The lower window margin contains the status bar that issues current experiment data (figure 69). From left to right these are: Analyzed frames per second (*Frame rate*), effective video position (time), experiment start date and time, experiment *Duration*, experiment stop date and time, *frames dropped* (count).


Configuration
Acquisition
Data analysis
0,0 cm
Fishy
0,0 cm
0,0 cm
Frame TC: 0:00:41,280
Head
Body
Tail
Show zone data
Reduced track
Results:
Fishy, Group Gr 1
Overall track length: 212,0 cm
Interval summary
No. | Date | Time | Avg. velocity (cm/s) | Track length (cm) | Activit
36 | 20.12.2007 | 14:43:51 | 5,3 | 5,1
37 | 20.12.2007 | 14:43:52 | 5,6 | 6,3
38 | 20.12.2007 | 14:43:53 | 6,4 | 6,1
39 | 20.12.2007 | 14:43:54 | 6,7 | 6,4
40 | 20.12.2007 | 14:43:55 | 6,4 | 6,1
41 | 20.12.2007 | 14:43:56 | 6,4 | 6,7
figure 68

The *'frames dropped'* item enumerates the total number of frames that were not analyzed during a live acquisition of the camera signal, due to a lack of processing power. Whenever the computer is incapable to process in real time, a frame has to be dropped in order to refit result and video time.

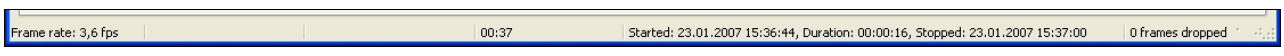


figure 69

Start data acquisition

The Run button

To start an experiment click the green *Run* icon in the vertical toolbar on the left oft the video image (figure 70). Subsequently the button changes color to gray and the *Stop* button becomes red, as in figure 68. The colored track of the experimental animal will appear in the video picture as soon as the animal moves. Data will automatically be logged in the corresponding tables and graphs.

figure 70

Time scheduled start

To learn about time controlled experiment start, see chapter *Configuration – Experiment.*

Zone visit triggered start

How to create zones for experiment start on zone entry or zone exit is described in chapter *Configuration – Zone Definition*. If a start zone is defined *Viewer* does not start acquisition immediately after the *Run* button click. It waits for the animal to enter or to leave the start zone. That depends on whether the *Start* or *Start on leave* trigger was set. While *Viewer* is waiting for the animal's action the green *Start* button is disabled while the red *Stop* button is enabled (figure 71), but no track is recorded. There is also a hint that *Viewer* is in "waiting status" in the software title bar.

The waiting status ends with acquisition start or when the red *Stop* button is operated.
You can use *Start* triggers in live acquisitions as well as in video file analyzing.

When you analyze a movie file, you may want to jump to a certain scene first. Use a *Movie bookmark* (see next chapter, *Video display options*) or the video control panel under the video display for targeted playing. Moving the video slide ruler updates the video time in the lower right corner of the image (figure 68, *Frame TC* means frame time code).

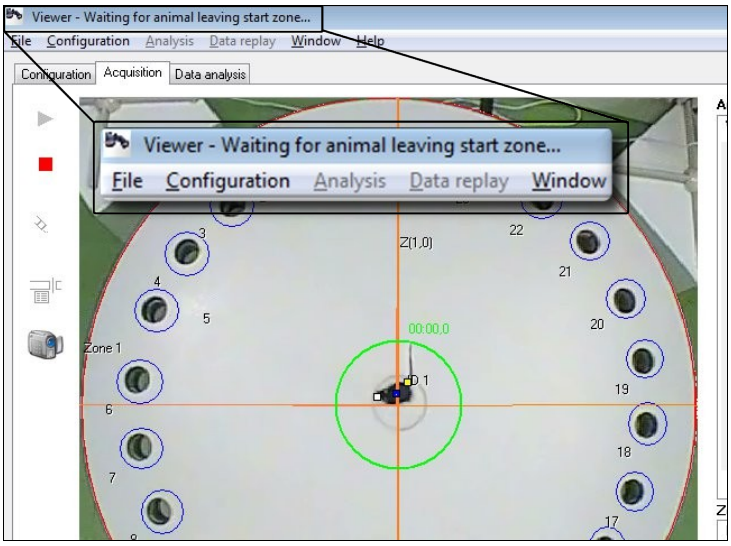

Viewer - Waiting for animal leaving start zone...
File Configuration Analysis Data replay Window Help
Configuration Acquisition Data analysis
Viewer - Waiting for animal leaving start zone...
File Configuration Analysis Data replay Window
Z(1,0)
Zone 1
00:00.0

figure 71

Video control panel (figure 68) functions:

Slide ruler	for targeted switching to a certain video time or sequence
Play / Pause button	starts, interrupts and restarts video playback
Stop button	stops video playing and resets the slide control to start position

Stop data acquisition

Acquisition can always be stopped manually by clicking the *Stop* icon in the vertical toolbar.

If you analyze a saved video and don't stop manually, acquisition stops automatically at the end of the footage.

To learn about further stop functions like time restriction or zone data dependency, see the foregoing chapters *Configuration − Experiment* and *Zone Definition*.

A *Save* dialog will instantly appear when acquisition stops. Please name and save the results. The target folder can be changed by manual browsing in the save dialog. The default path to the result files is:

..\Viewer\Data
'..\' is a placeholder for the path to your *Viewer* directory

Video display options

Switch on camera

The bottommost icon in the vertical toolbar is a video camera (figure 70). The function recapitulates the *Switch on* and *Switch off* buttons from the *Filters and Objects* page. It is located here to save time and clicks, in case you accomplish a whole series of similar experiments. The idea is, that you can start the *Viewer* and proceed to the *Acquisition* page immediately, without visiting the *Filters and Objects* page.

Erase track

During data recording the path covered by the animal is displayed in the video picture as a colored line. In long lasting experiments this may draw a confusing image after a while.
The pencil button with the eraser tip in the vertical toolbar (figure 70) can be used to erase the animal's track from the video image during acquisition. This has no effect on gathered data at all − it just clears the display to reveal the video image.

Reduced track

Instead of erasing the track every now and then, you can tick the *Reduced track* check box (figure 68). This activates an algorithm that constantly erases the track except the last 100 data points. The *Reduced track* option is checked by default.

Show zone data

If the *Show zone data* check box is activated, zones and zone-specific results are visible in the video image during acquisition. The depicted values resemble the selected parameter in the online statistics, as we will see soon.

Movie bookmarks

Movie bookmarks are time positions in a video. You can use them to jump to marked scenes by a single click. Defining a bookmark is possible whenever a video file is played-pack, no matter whether an acquisition is running or not. Open the *Movie bookmarks* window (figure 72) by selecting the *Movie bookmarks* item from the *Window* drop-down menu in the program main menu.

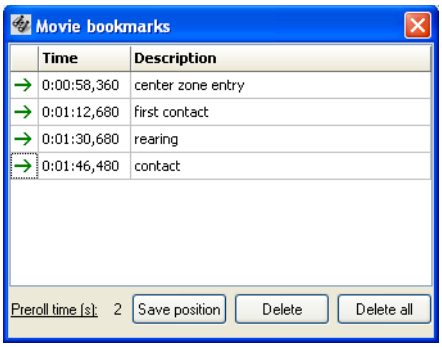


figure 72

Operate the *Save position* button to create a bookmark. A new bookmark will be added to the list in the *Movie bookmarks* window as table row. Into the *Description* field, you can enter something to recognize the scenes later. With clicks on the green arrows you can navigate through the bookmarked scenes.

It is useful to define a *Preroll time*, that is subtracted from the bookmark's time position. Otherwise you will jump into a scene without watching how it began. Click the *Preroll time* label to open a pop-up dialog. There you can enter a number to define the leader's duration in seconds.

At any time you can delete single or all table rows by the buttons *Delete* and *Delete all*. A certain bookmark is deleted, when it is marked or the cursor is in the respective line. Otherwise the last table row is deleted, when *Delete* is operated. All bookmarks are removed whenever a new video file is loaded. Closing the *Movie bookmarks* window doesn't deletes your entries.

Online statistics

Interval results table

Placed in the lower left corner you see the table that shows summed up interval data during data acquisition. The saving interval length was defined under *Configuration − Saving Options*. If you want to work with manually − perhaps event dependent − intervals, define the saving interval to exceed the experiment duration. Then you can use the *Create summary now* button from the vertical toolbar (fourth from the top, figure 70) to insert interval bounds during acquisition. There is one table for each animal, placed on single tab sheets. These sheets are labeled as follows: *Area name*, *Group name, Animal name* or *ID*.

Note: The online statistics on the *Acquisition* page show results for the selected tab sheet only. You can switch from one to another whenever you like to, even during acquisition (figure 73).

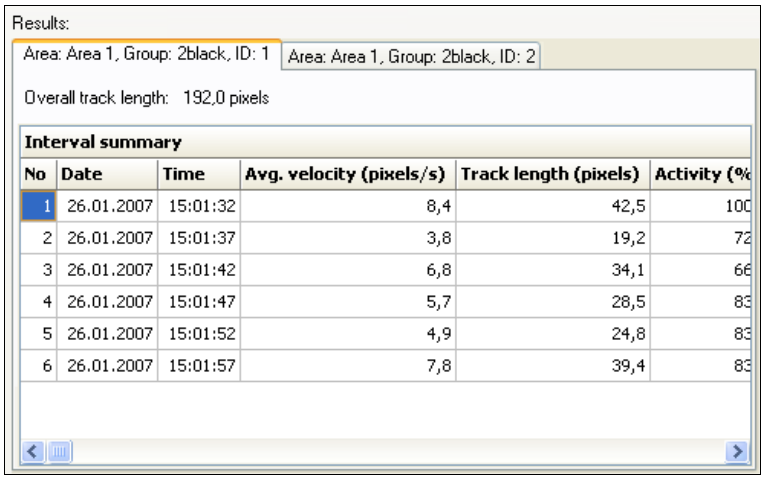


figure 73

All parameters are deduced from time and length measurements. The used unit of length is defined by the user in the process of calibration (see chapter *Configuration − Filters and Objects*). The table contains the parameters in the following list.

For the saving intervals:

No.	The interval number is assigned in chronological order
Date	Actual date of the end time of the interval in the format day.month.year
Time	Time of the day when the interval ended.
Avg. velocity	Average speed, measured in the whole arena
Track length	Total length of the track, measured in the whole arena
Activity	Amount of time in which the animal's movement speed exceeded the *Activity* threshold (user defined on the *Configuration − Activity* tab)
Ambulations	Total count of accelerations from inactivity to a speed, faster than the set *Ambulation* threshold (user defined on the *Configuration − Behavior* tab)
ZC	Total count of *Zone crossings* (user defined on the *Configuration − Activity* tab)
RZC	Total count of Rated z*one crossings* (user defined on the *Configuration − Activity* tab)
Wall dist.	Distance of the animal's body point to the nearest wall, averaged over all video frames (the wall is user defined on the *Configuration − Activity* tab)
Area covered	The covered area is another issued activity parameter. It is the percentage of visited grid cells in the whole area. These cells refer to the user defined *Zone crossing* grid. The reference pane is the *Wall zone* area. Grid and Wall zone are defined under *Configuration – Activity*)

For *Velocity classes*:

vc1 to *vc10*	Time the animal spent moving with a speed according to a *Velocity class* (user defined on the *Configuration − Activity* tab)

For each zone:

Duration	Total time the reference point stayed in a zone. [1]
Velocity	Average speed, measured during the *Duration of stay*
Track length	Total length of the track, measured in during the *Duration of stay*
Visits	Total count of zone entries by the reference point(s)
Headbobs, Tailmoves, Headstretches, Freezings	Total count of the specific behavior during the *Duration of stay* (only visible if the respective detection algorithms are activated and user defined on the *Configuration − Behavior* page)
Activity	Amount of active time during the *Duration of stay*
Ambulations	Total count of *Ambulations* in the zone during the *Duration of stay* (only visible if activated)
Visit latency	Elapsed time to the zone entry since the saving interval started

[1] Duration values are always rounded down! 3.79 s and 3.45 s are issued as 3.7 and 3.4, in order not to produce sums of durations that exceed the total experiment time.

Area data

During the experiment acquired data are presented in various graphs and bar charts in the right window half. The graphs in the upper chart assembly visualize tracking-area-data per saving interval. At the end of each saving interval a data point is added or bar charts are updated. The only exception is the *Real time* mode of the velocity tab (figure 74).

Velocity:

The animal's locomotion velocity is presented on the outer left tab in the upper chart assembly. In the right corner under the chart you can select by buttons how you want the aspect to develop. The Real time graph is drawn in a diagram with a fixed (but manually resizeable) scale on the vertical axis and a dynamic time scale on the abscissa. Here data is updated in every single video frame. Above the y-axis there is a button to adjust the vertical scale's maximum value manually by using a pop-up slide ruler.
Two different characterizations for the Real time graph are at hand. One with plateaus between the break-points and one with pointed maximums and minimums.
You may want to compare the effective data with your defined thresholds for *Activity* and *Ambulation* (see chapter *Configuration – Activity* and *Behavior*). You can superimpose these corresponding vertical lines in the chart. Therefore activate the check box *Show Activity/Ambulations* in the left corner under the chart.

The *Complete* view resumes interval sums. After each finished interval, a new data point is added to the abscissa, whereas the y-axis adopts the highest measured value so far. (compare figure 76 and figure 78).

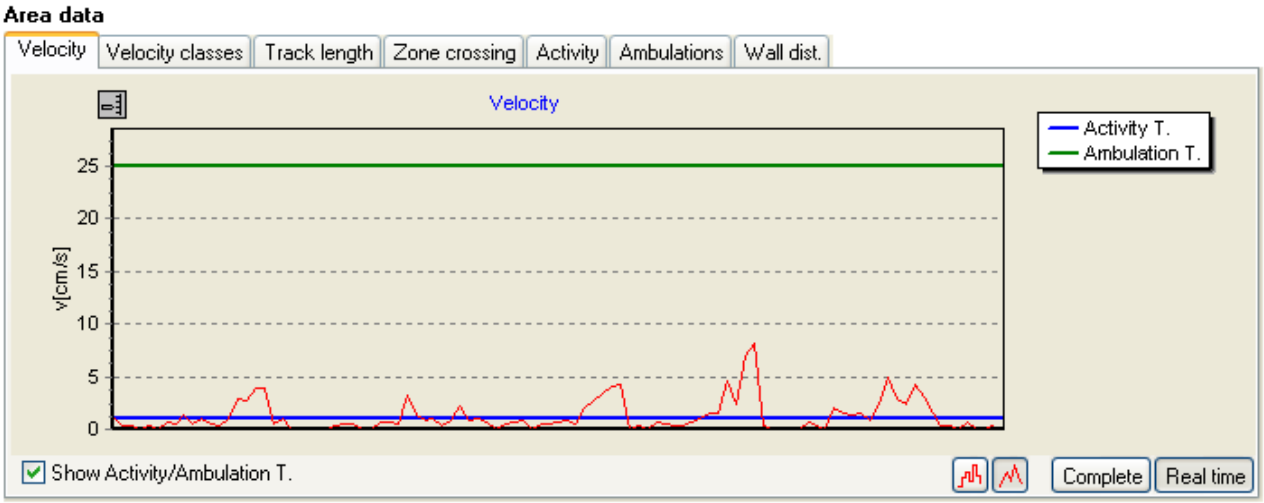


figure 74

Note: In the *Complete* mode of the *Area data - Velocity* tab, you will see no results until the first saving interval is finished.

Configuration | Acquisition | Data analysis

Velocity classes:

Clicking *Velocity classes* reveals a column diagram of the distinct velocity classes. A columns height indicates the duration of stay (in seconds) of the animal within the specific class. This chart is updated each time a saving interval ends.

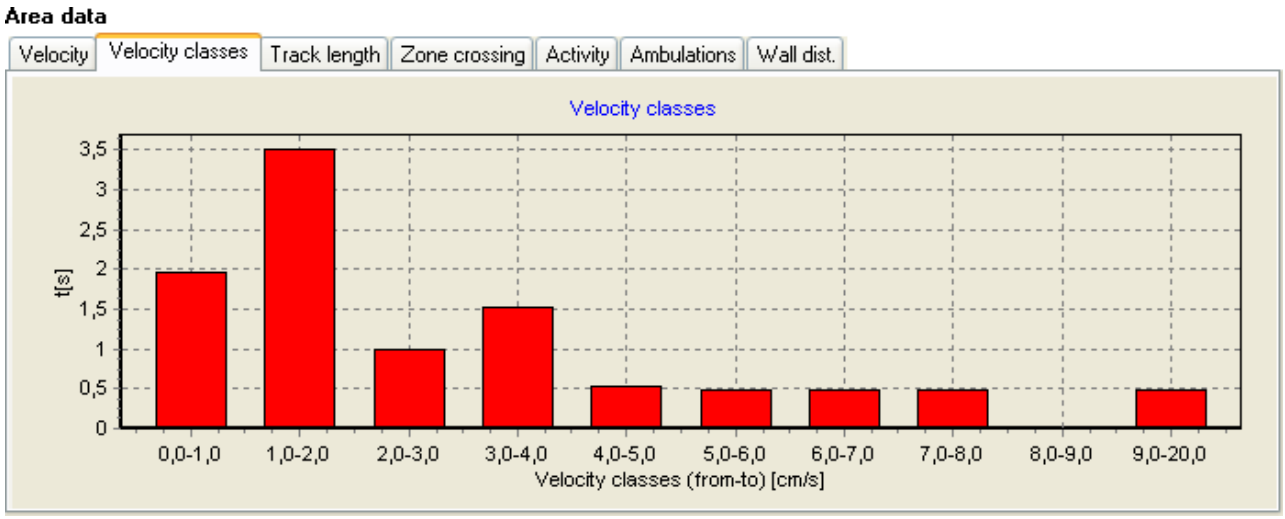


figure 75

Track length, Zone crossing, Activity, Ambulations:

These diagrams are all of the same kind and comparable to the C*omplete* view of the *Velocity* parameter. The *Zone crossing* graph offers the additional option to choose between rated and unrated zone crossing. The respective buttons are located under the chart in the right corner.

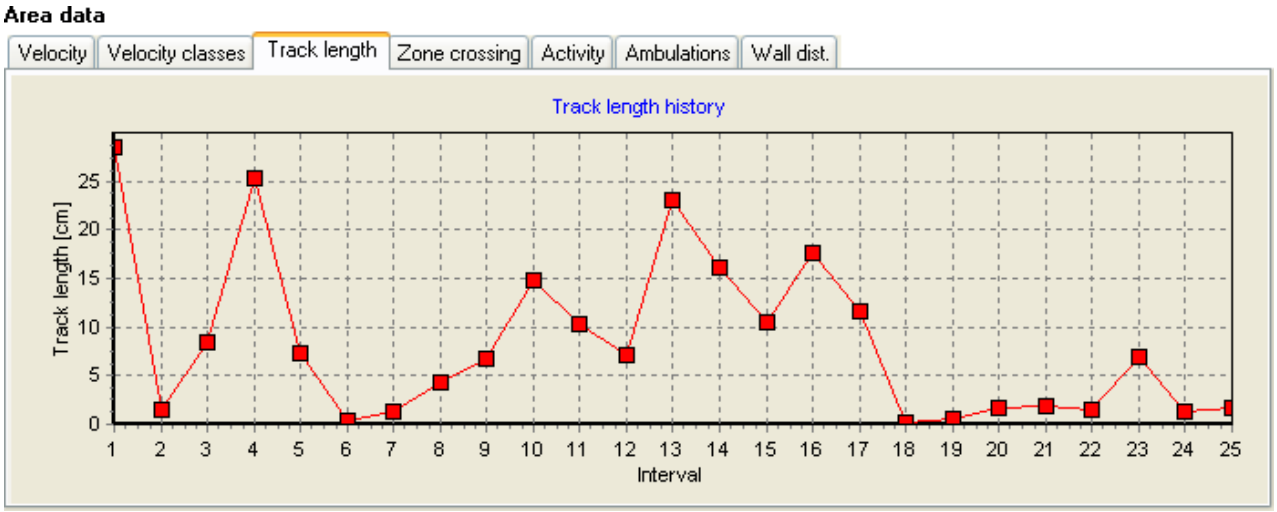


figure 76

Zone data

Interval view

The charts and sketches in the middle of the right window half visualize zone data. Use the tabs to switch from one parameter to another. Use the *Complete* and *Interval* buttons to switch from graphs to sketches. The sketches show exactly the layout of tracking areas and zones, you set up in *Configuration* mode. The interval results for each zone is displayed in stead of the zones' names.
During the experiment the zone based values for *Duration*, *Velocity*, *Track length* etc. sum up until a saving interval ends. All values are reset to zero at an interval end, and they begin to increase again during the next interval − presumed there is data to sum up. If you did not divide the arena in zones before, a value renders the entire experimental arena.

figure 77

Activating the *Show background* check box bolsters the sketch with the first video frame, respectively the first recorded image. That's a nice tool for screen shots. You can present data on a picture of the real setup. The tool is at hand here and on the *Data Analysis* page. There you will be able to display the summed up results for zones, too.

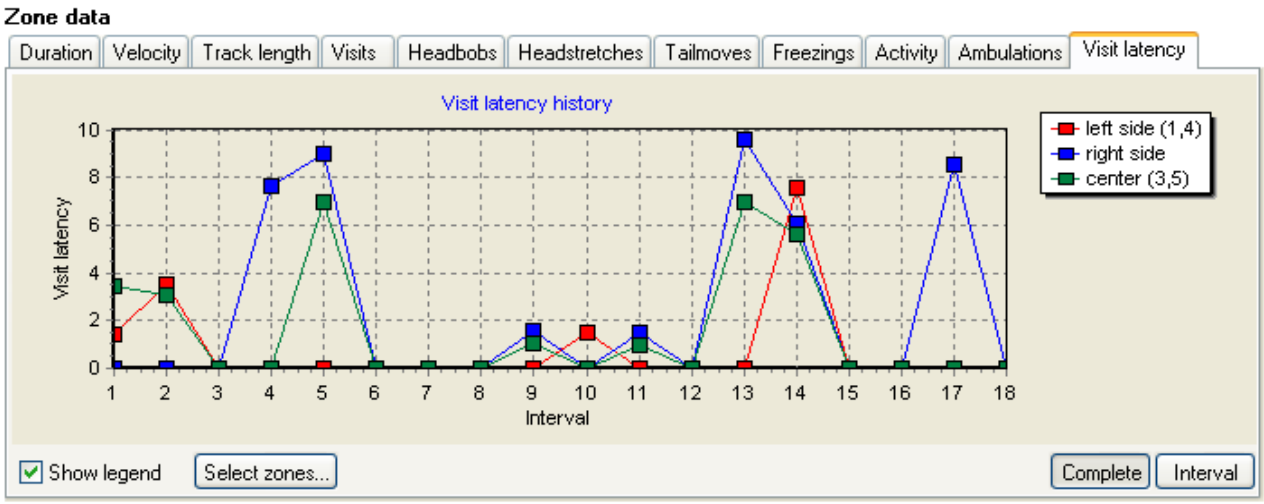


figure 78

Complete view

In *Complete* mode (button left from the *Interval* button) interval results for the selected parameter are shown consecutively until acquisition stops. The diagrams are built up and updated exactly like they are in the *Complete* mode in the upper chart assembly. But there is an additional feature regarding the choice of content.

As these diagrams show zone data you can select the zones you want to expose. Push the button *Select zones...* under the diagram. A small pop-up window occurs that offers a check box for each zone. Data for checked zones will be shown, unchecked will not. If you singled out some zones and want to return to the full view, you can always use the *Show all zones* button in the same window, to close the window and show all of them.

Note: Diagrams in *Complete* mode look similar for *Zone data* and *Area data*. Each data point stands for a result at the end of a saving interval. The bar chart on the *Area data – Velocity classes* tab shows interval results as well. All other *Area data* charts are depicted in complete mode. Switching between *Interval* and *Complete* mode is only possible on *Zone data* tabs.

Raw data

Below the zone data statistics, the track points' positions are displayed in a table. A blue row indicates the current data points. Because these positions are saved in the *Viewer* result file, new parameter calculation on old data is possible. See figure 103 and *Reanalyze current file* in chapter *File menu*.

Data Analysis

Results – Result Export – Experiment Review – Track Correction – Data Correction – Statistics

The analysis functions of the program include an experiment playback, various graphs based on the acquired data and a tabular summary of the data. As you know the *Acquisition* mode by now, the *Data Analysis* page will look quite familiar to you. It is constructed with the same main elements, so we can confine this chapter to the description of exclusive data analysis features.

Results

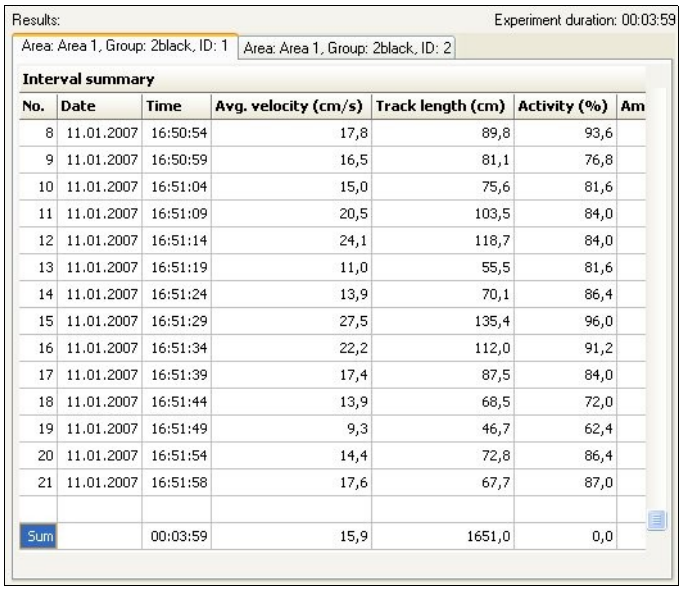
Below the video review tools you find a tabular summary of the experiment data that corresponds to the *Results* table on the *Acquisition* page. The last interval data row is now followed by an empty row, than by one that contains total sums of the interval results. Above the table the total *Experiment duration* is indicated.

The *Sum* row at the table's end resumes the saving intervals' data. It contains averages, sums and a individual values:

Sums	*Time (total duration), Track length, Ambulations, ZC, RZC, Velocity classes, Visits, Headbobs, Headstretches, Tailmoves, Freezings,*
Averages	*Velocity, Activity, Wall dist.*
Single value	*Visit latency*: Here the first zone entry per total experiment time is issued

Result export

If you cancel the save dialog after the experiment but decide to save the data later, click the disk icon (*Save results*, figure 81) in the vertical toolbar on the left from the video display to reopen the save dialog. The data will be saved as *Viewer* result file (*.vrf).
The other two buttons in the vertical bar cause data exports. You can either export in *.csv format or in Microsoft Excel format (*.xlsx). An advantage with Excel is, that you attain a portfolio with sheets. Each containing one animals results (similar to the *Viewer Result lists)*.


Results: Experiment duration: 00:03:59

Area: Area 1, Group: 2black, ID: 1 | Area: Area 1, Group: 2black, ID: 2

Interval summary

No.	Date	Time	Avg. velocity (cm/s)	Track length (cm)	Activity (%)	Am
8	11.01.2007	16:50:54	17,8	89,8	93,6	
9	11.01.2007	16:50:59	16,5	81,1	76,8	
10	11.01.2007	16:51:04	15,0	75,6	81,6	
11	11.01.2007	16:51:09	20,5	103,5	84,0	
12	11.01.2007	16:51:14	24,1	118,7	84,0	
13	11.01.2007	16:51:19	11,0	55,5	81,6	
14	11.01.2007	16:51:24	13,9	70,1	86,4	
15	11.01.2007	16:51:29	27,5	135,4	96,0	
16	11.01.2007	16:51:34	22,2	112,0	91,2	
17	11.01.2007	16:51:39	17,4	87,5	84,0	
18	11.01.2007	16:51:44	13,9	68,5	72,0	
19	11.01.2007	16:51:49	9,3	46,7	62,4	
20	11.01.2007	16:51:54	14,4	72,8	86,4	
21	11.01.2007	16:51:58	17,6	67,7	87,0	
Sum		00:03:59	15,9	1651,0	0,0	

figure 79

Note: Excel can build tables with up to 250 columns. If you have more than 20 zones in your configuration, your result table inside *Viewer* will be larger than an Excel table can be. Trying to export to Excel format will produce a bug report with an exception message: Invalid Column (256). In this situation you can use the button *'continue application'*. *Viewer* will work on correctly.

Experiment review

In the top left corner of the *Data analysis* page the track is displayed.
While the *Acquisition* page always shows the live video signal in this position, the *Data analysis* page can either replay the experiment video or it simply shows the first captured frame of the experiment as background for the track. The background picture is saved inside the *Viewer* result file. Thus it is always visible when a result file is opened.
On the right side of the display is the green *Show/Hide video* button. Click the button once to hide the video picture and thereby to extend the space for the *Result* table. Click it once again to show the video picture.
The experiment video is saved separately in an AVI file. It can be replayed if the file is located in the same folder like the result file and has the exactly the same name. For example:
experiment-01.avi and experiment-01.vrf

figure 80

Play, pause and stop in the video control panel under the display control the video. If no video file was loaded the panel replays an animation of the track points only.

- Activating the *Slow motion* check box under the video control panel slows down playback speed.
- *Show zone data* switches zones in the main video display (figure 80) on and off.
- Directly under the display you can read the *Visible track segment:* label. The button next to it opens the dialog in figure 81. The purpose of this dialog is to adjust the length of the reduced visible track during replay. Alternatively you can set it to *Overall* to show the complete track.

Note: While during acquisition all tracks are displayed, here the track for only one animal is shown if two or more animals were observed. Due to the fact that these tracks are results now, this is consequent. All visible statistics and the track refer exclusively to the selected animal's result table. There is one tabbed table sheet for each animal (figure 79).

Track visualization

Similar to the *Acquisition* page you can switch between head, body and tail track for visualization. By default the *Body* point track is visualized. This is indicated by the bold font of the label in the color key below the video (like in figure 80). If you want to visualize another track, simply click on the respective track point label.

figure 81

You can navigate to a special position to see how track data was interpreted. Navigation is facilitated by the *Time* information below the slide ruler. *Time (relative)* represents the relative time of the experiment, whereat acquisition start is the initial point in time. *Time (absolute)* shows the date of the experiment, followed by the real time of the day during the experiment.

A blue bar scrolls through the result table during playback or whenever you move the video control panel's slide ruler manually. It highlights the actually visualized time bin of the experiment. Data is synchronously visualized in the *Zone data – Interval* charts (figure 87) and in the *Area data – Velocity classes* chart on the right (the other *Area data* charts show the complete experiment history).

It is also possible to mouse click into the table directly to select an interval result manually. Combined with the additional display options, explained later on in this chapter, you can show the animal's track in each saving interval.

The table shows different possibilities to visualize the track

Visible track segment setting	Video display	*Zone data* chart (*Interval* mode)
Overall	Complete experiment track.	The complete track of the highlighted interval. Regardless the video slider ruler's position. *Show track* has to be activated (figure 87). The *Sum* line of the result table can be highlighted as well to show the compete track.
Relative to current time position	Track that covers the user-defined time span relative to the video slide ruler's position. Regardless the highlighted interval in the result table.	

Track correction

The *Track correction* window is an interface to edited raw data. You can move points you regard as misplaced or artifacts. Like track spikes to reflections.
The pencil tool in the vertical toolbar on the left side of the video display (figure 80) opens the *Track correction* window.
Each data point in the track is a blue square. Figure 82 shows a complete track. Obviously it is hard to identify a single point of interest in these heaps of track points.
For a better overview you can first select an interval in the *Results* table to reduce the amount of depicted track points. Then open the *Track correction* window and finally zoom in.
Figure 84 shows an interval track of the same experiment. There it is quite easy to discern unwanted track points.
A selected square changes color to white. Selecting several points at once is possible, too. Activate the pointer tool and draw a square to select all track points within the outlined area (figure 83). With the pointer tool selected track points can be dragged and dropped by holding and releasing the left mouse button.
With the magnifier tool you can zoom in.
The button with the full-screen-icon (below the hand icon) resets the zoom level to 100%.
With the hand tool a zoomed image can be snatched and shoved around to navigate along the track.

The buttons *Head*, *Body* and *Tail* stand for the three recorded animal tracks. You can edit each of them and switch between them with those buttons.
To implement your changes in the track close the window with the *Close and reanalyze* button. The new track points' coordinates will be written into the raw data and all track parameters will be recalculated.
With the *Clo*se button the window can be left without reanalyzing.

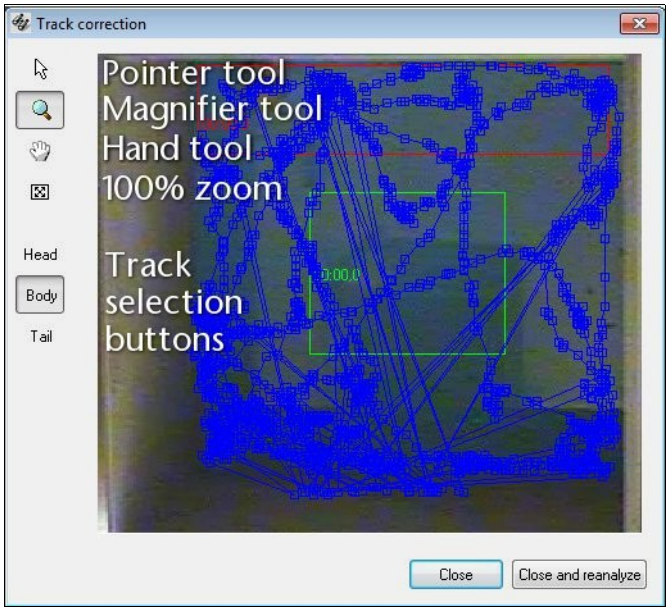


figure 82

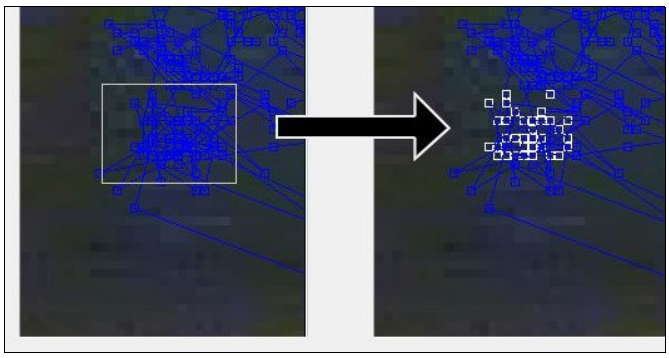


figure 83

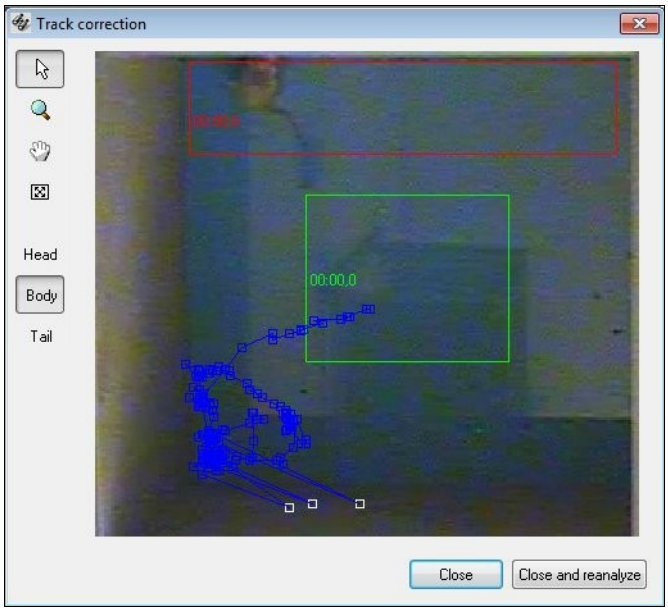


figure 84

Trim data

This function takes into consideration that an experiment movie might show a person putting an animal into the setup at the beginning and takes it out at the end. Such a movie can be analyzed either by manual acquisition start and stop, or by cropping the data after acquisition.
To open the *Trim data* window, select the *Trim data...* item from the *Analysis* drop-down menu in the program main menu. Enter the appropriate periods of time into the two input fields labeled *at the beginning* and *at the end*. Then, confirm with the *Close and reanalyze* button to attain the cropped result.

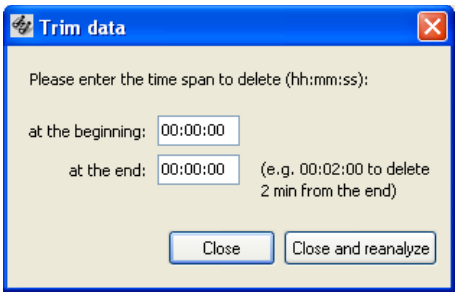


figure 85

Statistics

The charts show the same content as on the *Acquisition* tab and they have nearly the same functionalities.
But there are some extra features on the *Data analysis* tab, that will be discussed here.

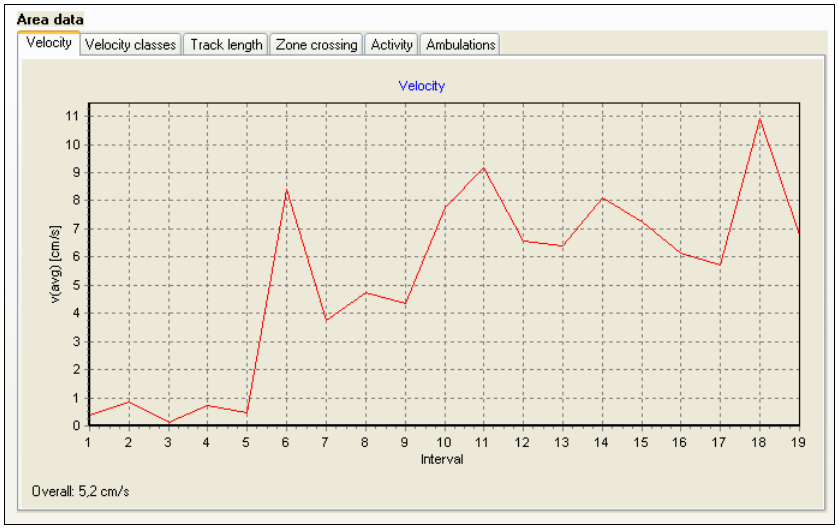


figure 86

Overall parameter results

On the *Data analysis* page an additional *Sum* line (figure 79) is appended to the *Results* table. In the lower left corner of each *Area data* diagram (figure 86) the respective sum or average of the parameter is shown as *Overall* value.

Fixed Y-axis scale

In the *Area data* diagrams the y-axis scale is automatically set to to the highest measured value plus 15 %. To compare *Area Data − Velocity classes* charts from different experiments you can activate the check box *Fixed scale* under the chart. This betters comparability of results from simultaneously observed arenas. Because the scale does not change when you switch between experiments.

Interval and complete Zone data results

In *Interval* mode (*Zone Data*) the sketches show interval data for the interval selected in the *Results* table. Selection is done by clicking an arbitrary field in the table. The field becomes highlighted and the statistics update immediately. Only the vertical position of the highlighted field determines the interval − column choice has no effect on the statistics. As you now have the *Sum* row at hand, you can choose to display the total sums as well in *Interval* mode.

Additional display options

Additional display options are placed beside the *Show background* check box. First, there is the *Show track* option. Based on the gathered raw data, the animal's track can be mapped for each saving interval (figure 87 shows the track of only one interval). The interval highlighted in the *Results* table is shown exclusively. But it is possible to show the whole track, like in figure 89, as well, if the S*um* row is marked in the *Results* table.

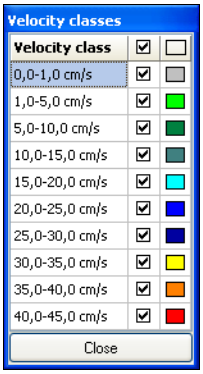


figure 88

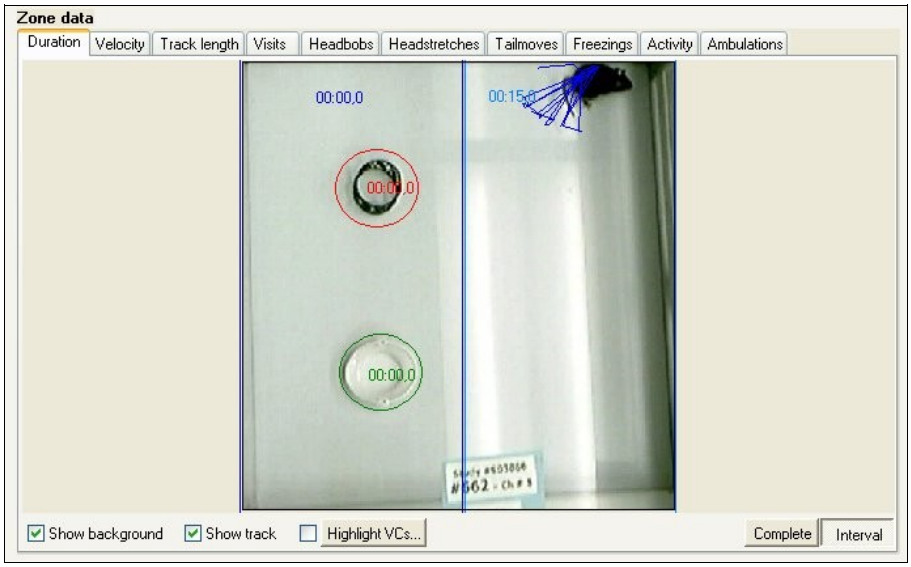


figure 87

The ***Highlight Vcs...*** button opens a color selection window (figure 88) where you can adjust colors for parts of the track according to their *Velocity class* membership. The check box activates this option. And of course, the *Show track* check box has to be checked therefore too.

You may chose the same color for several classes to make the sketch easier to understand. This was done to survey the track in figure 89, where only three different colors were used. The result is, that the lower classes produced green bundles that stand like markers for the resting points of the animal. The few red and blue lines indicate some fast area crossings.
All classes can be reset to the same color by adjusting the uppermost field in the little pop-up window.

figure 89

Data replay

By default *Viewer* doesn't show the *Data replay* page. You need to start it with a prepared desktop short-cut. The short-cut has to contain a start parameter that is added to the file path in the short-cut properties:

"C:\Program Files (x86)\BIOBSERVE\Viewer\Viewer.exe" /enabledatareplay

The path The *Data replay* interface offers valuable insight into acquired data. It is especially useful for finding the best behavior recognition settings and to investigate on special events in long lasting experiments. In contrast to the *Data analysis* tab, here result presentation is focused on the time line, not on statistics visualization.

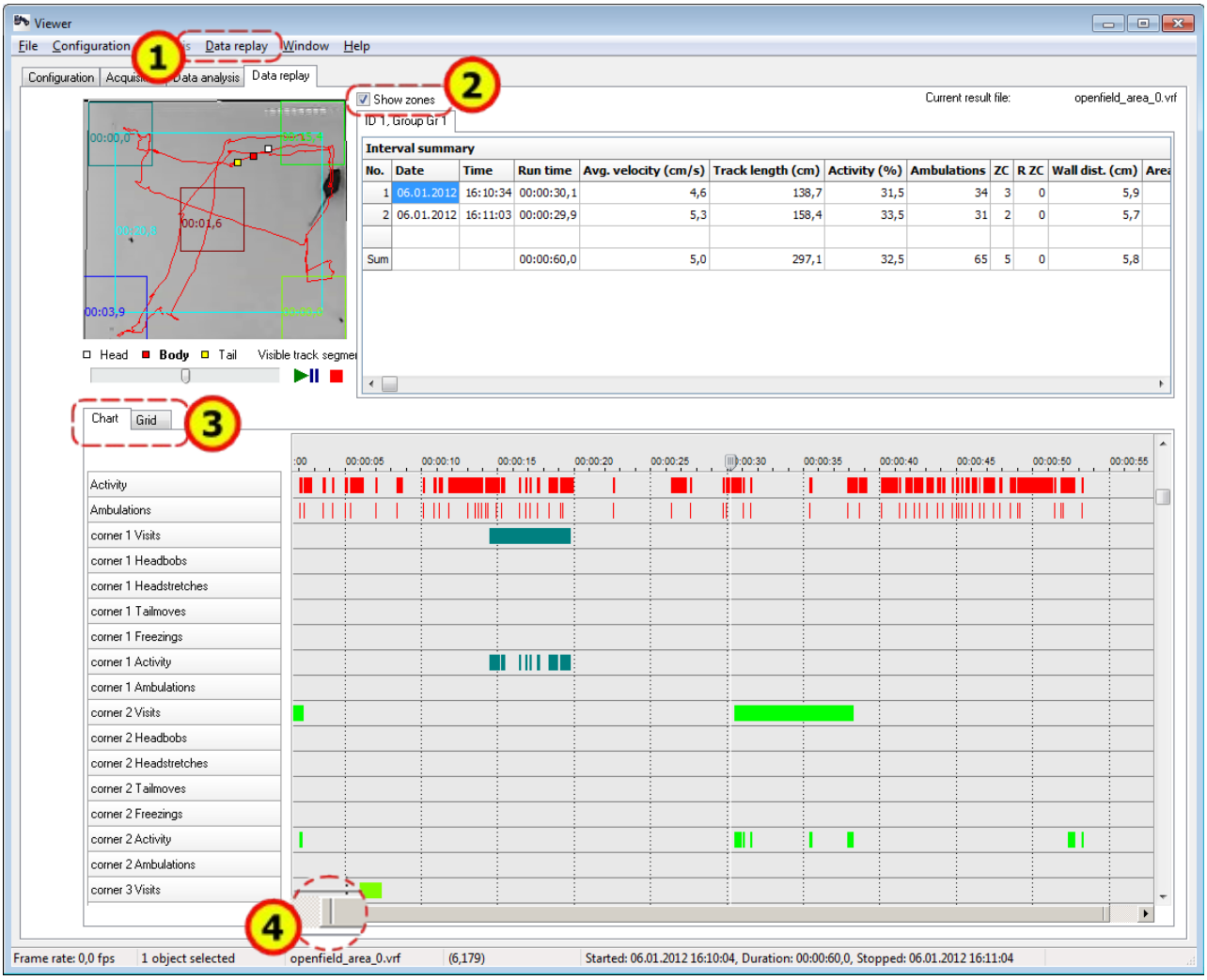


figure 90

There are two ways to navigate through the experiment. First, you can replay the video by using the video slide ruler, the play, pause and stop button under the video. The position indicator in the ethogram below makes synchronous moves on the data time line. The ethogram shows *Area data* and *Zone data*.

You can also drag the position indicator to a new position in the ethogram. Or simply mouse-click on the ethogram position you are interested in. For example to watch the video sequence of a detected behavior. The video jumps to the respective scene in the video instantly.

By changing the zoom level you are able to assess logs with short duration even in long lasting experiments. To zoom in the ethogram hold and drag one of the marks on the left and right border of the lower horizontal slider (figure 90 enlarged element 4).

Similar to the *Acquisition* tab and the *Data analysis* tab you can switch zone visibility on and off (figure 90 element 2).

From the *Data replay* menu (figure 90 element 1) you can export the ethogram as BMP image file and the *Grid* (figure 91) as CSV or Excel file. Switch between *Grid* and *Chart* view with the tabs in figure 90 element 3.

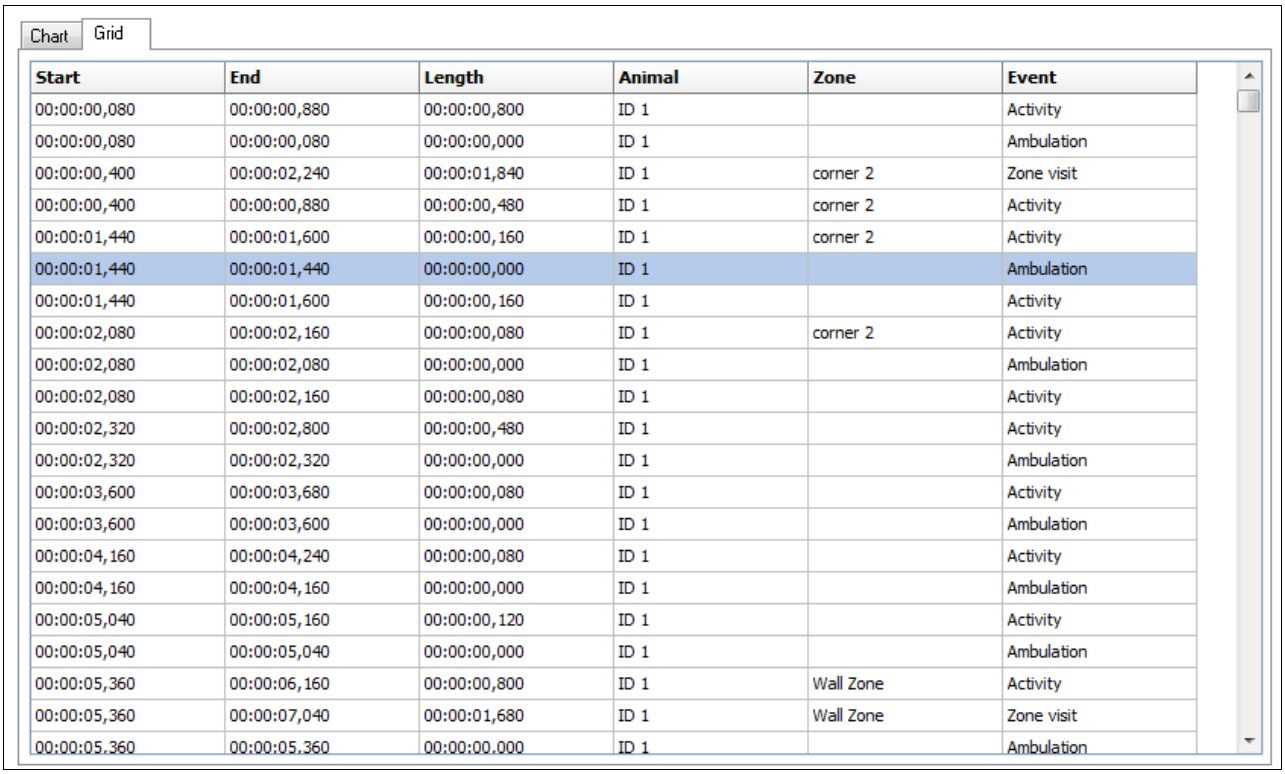


Chart | Grid

Start	End	Length	Animal	Zone	Event
00:00:00,080	00:00:00,880	00:00:00,800	ID 1		Activity
00:00:00,080	00:00:00,080	00:00:00,000	ID 1		Ambulation
00:00:00,400	00:00:02,240	00:00:01,840	ID 1	corner 2	Zone visit
00:00:00,400	00:00:00,880	00:00:00,480	ID 1	corner 2	Activity
00:00:01,440	00:00:01,600	00:00:00,160	ID 1	corner 2	Activity
00:00:01,440	00:00:01,440	00:00:00,000	ID 1		Ambulation
00:00:01,440	00:00:01,600	00:00:00,160	ID 1		Activity
00:00:02,080	00:00:02,160	00:00:00,080	ID 1	corner 2	Activity
00:00:02,080	00:00:02,080	00:00:00,000	ID 1		Ambulation
00:00:02,080	00:00:02,160	00:00:00,080	ID 1		Activity
00:00:02,320	00:00:02,800	00:00:00,480	ID 1		Activity
00:00:02,320	00:00:02,320	00:00:00,000	ID 1		Ambulation
00:00:03,600	00:00:03,680	00:00:00,080	ID 1		Activity
00:00:03,600	00:00:03,600	00:00:00,000	ID 1		Ambulation
00:00:04,160	00:00:04,240	00:00:00,080	ID 1		Activity
00:00:04,160	00:00:04,160	00:00:00,000	ID 1		Ambulation
00:00:05,040	00:00:05,160	00:00:00,120	ID 1		Activity
00:00:05,040	00:00:05,040	00:00:00,000	ID 1		Ambulation
00:00:05,360	00:00:06,160	00:00:00,800	ID 1	Wall Zone	Activity
00:00:05,360	00:00:07,040	00:00:01,680	ID 1	Wall Zone	Zone visit
00:00:05,360	00:00:05,360	00:00:00,000	ID 1		Ambulation

figure 91

Program main menu

The main program menu is placed under the title bar on top of the window. It helps you to administer your files containing results, raw data, configurations, movies, diagrams, pictures and sketches. It offers help and program specific commands. Some commands are already discussed and may be familiar to you, because they can be executed with buttons directly from the graphical interface as well.

File menu

Result files

Here is the place where you save, copy and – if necessary – reprocess results. Activate the *open* dialog to look for *Viewer* result files on your computer (***Open results...***).
In case the result file contains plug-in specific data but the respective plug-in is not active at the very moment, you will be warned by a pop-up window (figure 93).
Rest the mouse over ***Open recent results*** > to expand a list that offers the last ten used result files for quick launch. In this list result names are followed by "*(unavailable)*" if the file was deleted ore moved to another directory.
The ***Save results as...*** item opens the *save* dialog where you can (re)name the current result file (*.vrf) and save it. This way you can produce a copy of an existing *Viewer* result file or catch up on saving, if you canceled the save pop-up dialog after the experiment. The ***Export results...*** function offers result saving in Excel format or in csv format.
Please read the note concerning Excel export on page 46.

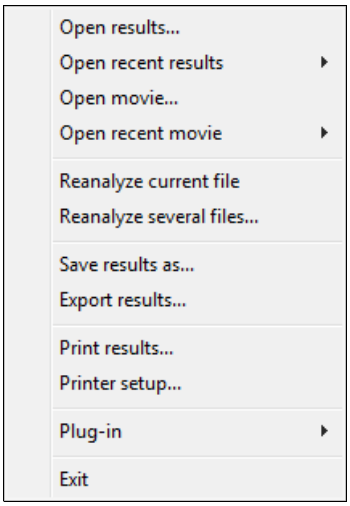


figure 92

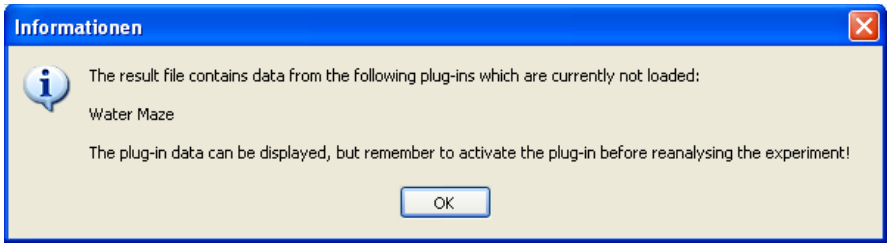

Informationen
The result file contains data from the following plug-ins which are currently not loaded:
Water Maze
The plug-in data can be displayed, but remember to activate the plug-in before reanalysing the experiment!
OK

figure 93

Data reanalyzing

You may find it useful to survey new parameters (zone visits, wall distance, reference point definition, etc.), with already existing track data. The
Reanalyze current file
item offers the possibility to reprocess existing data with a new configuration. Aside from that, reanalyzing can be used to send data to an interface, like a plug-in or a database.

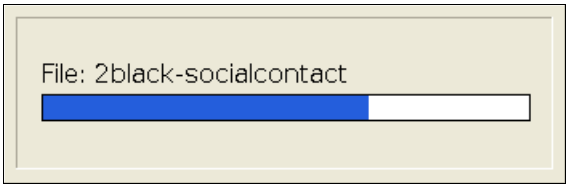


figure 94

When reanalyzing there is no need to run the experiment video again: Thus reanalyzing is processed very fast.
To reanalyze a single file proceed as follows:

- Open an existing result file.
- Change (and save) settings in the configuration or open an existing configuration file.
- Select the *Reanalyze current file* item from the *File* menu (figure 92).
- Save the new result, when reanalyzing is finished (indicated by a progress bar, figure 94).

Batch reanalysis

Similar to the *Reanalyze current file* item, you can reanalyze a batch of files at once with
Reanalyze several files...
The whole procedure is assisted by check back dialogs. First an *open* dialog will appear where you select an arbitrary number of files (figure 95). Subsequently a destination folder for the new files is determined (figure 96). In the last step you have to decide whether reanalyzing shall be conducted with the currently loaded configuration or with the original configurations of the single result files. A pop-up window asks for the method to use (figure 97).

- Select the *Reanalyze several files...* item from the *File* menu (figure 92).
- All files you want to reanalyze have to be located in the same folder. Use the *open* dialog (figure 95) to browse to the folder where you saved the files.
- Mark every file you want to select. Alternatively it is possible to select all elements in the folder by using the keyboard shortcut ctrl+A.
- If you want the *Viewer* to reanalyze the files in a special order, you can sort the files in the *open* dialog by name, size or date. As this dialog is an MS Windows component, you have to switch to the *details* view by the respective Windows button (red encircled element in figure 95). Click a column header to implement sorting.

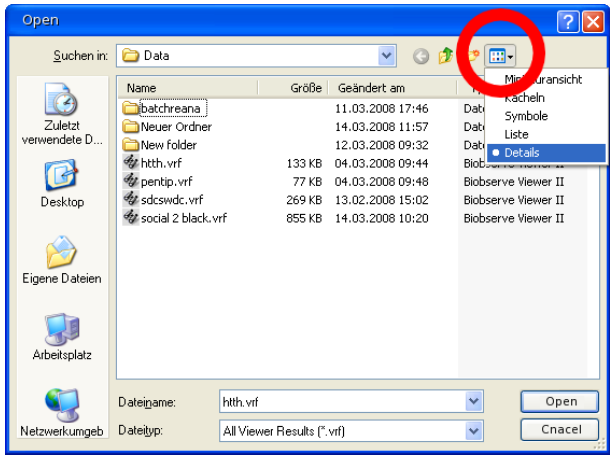


figure 95

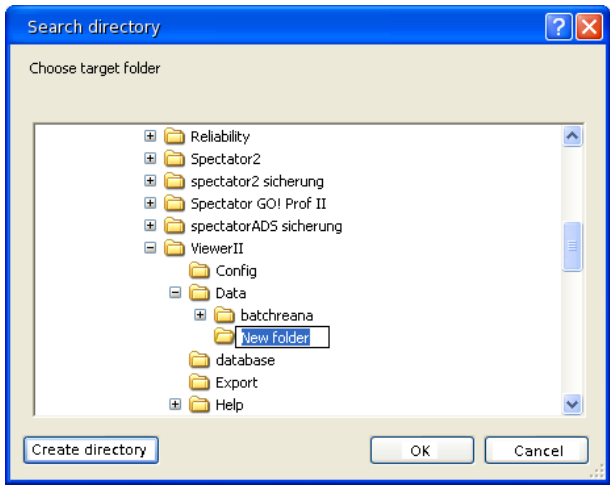


figure 96

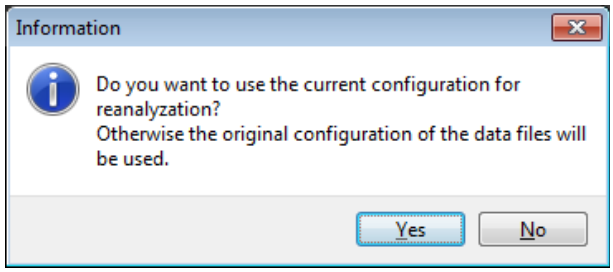

Information
Do you want to use the current configuration for reanalyzation?
Otherwise the original configuration of the data files will be used.
Yes
No

figure 97

- Confirm the *open* dialog with the *Open* button.
- In the next dialog (*choose target folder*, figure 96) the target folder has to be defined. Browse to an existing folder, highlight it and confirm with *OK*. Otherwise create a new folder by clicking the *create new folder* button in the lower left corner of the window. With the F2 key on your keyboard you can rename a selected folder (MS Windows standard function). When you're done, confirm with *OK*.
- The third and last dialog (figure 97) asks you, whether you want to use the currently loaded configuration for reanalyzing all files, or the configurations the files were originally produced with. Confirm with *Yes*, to use the currently effective configuration for all files. Confirm with *No*, to use the configurations each file was originally produced with. The second procedure is useful if you want to create new plug-in data from experiments you performed before you owned a specific plug-in, or if you forgot to activate the plug-in before acquisition.
- From now on data reprocessing and file saving happen automatically. While files are processed a list with progress bars for each file (figure 98) is shown.
- A currently used result file (before starting batch reanalysis) may be part of the list of processed files. If that is so *Viewer* asks whether or not to load the reanalyzed result at the end of the batch reanalyzing process (figure 99).

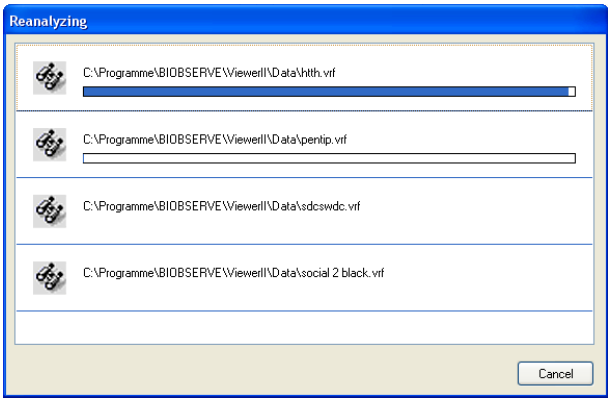


figure 98

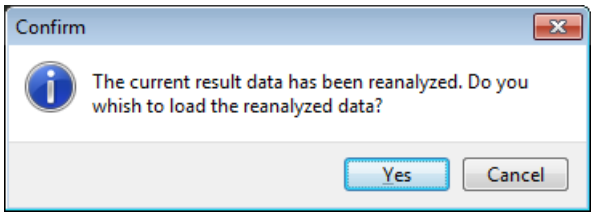


figure 99

Movie files

Open movie... activates the *open* dialog to look for movie files on your computer. The chosen movie will be displayed in the *Filtered picture* area on the *Filters and Objects* page.
Rest the mouse over ***Open recent movie*** > to expand a list of the last ten used movie files for a quick launch.

Result printout

Printer setup... opens the *print setup* dialog. The implemented setting affects the ***Print results...*** item. There you will find a preview on the printed result table where you can page up and down through the table and actuate a print job. For a customized table design you can print your exported data just as well.

Plug-in

A list of all installed plug-ins unfolds under the ***Plug-in*** > item (figure Fehler: Referenz nicht gefunden). Selecting a plug-in upgrades the *Viewer* with additional tabs that provide enhanced data analysis capabilities for specific experiments. All purchasable plug-ins are listed at the documents end and on our homepage under:
Products > Viewer > Plug-Ins.

Configuration menu

The last saved configuration is always loaded on program start. Configuration file administration is explained in detail in chapter *Configuration – Configuration files*. The menu commands resemble the File menu commands for *Viewer* result files (see previous chapter).

Note: Configurations are not saved automatically. Although you can work with an unsaved configuration and use it for acquisition, it will vanish when the program is closed.

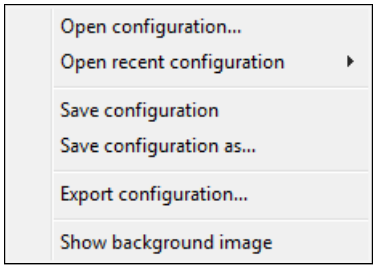


figure 100

All the rulers and input fields in the *Viewer* interface that contribute values to the setup configuration are placed on several different tabs. For publishing purpose you might need an abstract of these settings. For a simple list of configuration parameter settings use ***Export configuration...*** and select plain text (*.txt) as target format.

When saving a *Viewer* result file (*.vrf) a copy of the used configuration is saved inside the file. This result file internal configuration is loaded when a result file is opened.
Result files also contain a snapshot of the experimental arena in which the experiment took place. Sometimes it is preferable to take a look at the original setup, when reviewing former results.
Show background image > on/off switches the video aspect from the current video or camera picture to the image saved in the loaded result file.

Analysis menu

Graphics and sketches

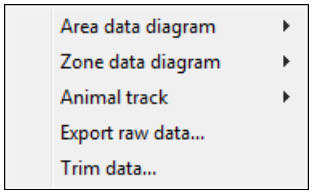


figure 101

The *Analysis* menu refers to items on the *Data analysis* page. It is concerned with different ways of output of the presented data. It is possible to ***Copy***, to ***Print...*** and to ***Save*** diagrams and sketches that appear in the *Zone data and Area data* boxes. The menu items *Zone data* and *Area data* contain these commands. They refer to the currently visible diagrams.
Example: To copy a diagram to the clipboard that depicts velocity values for zones in all intervals, select the *Velocity* tab in the *Zone data* box. Make sure that the *Complete* button is locked – instead of the *Interval* button. Next, navigate the *Analysis* menu to the command ***Zone data*** > ***Copy***. Now content can be pasted from the clipboard to another application. The same procedures works with all graphics and with ***Area data*** as well.
Save as... offers saving in the following file formats: Bitmap (*.bmp), Windows Metafile (*.wmf), and Enhanced Metafile (*.emf). This option allows you to insert the graphics easily in another program for further processing and publishing.
Print... opens the printer setup dialog where you can dispose a print job for the chosen graphic.

Track

Animal track > is a tool to ***Copy*** / ***Save as...*** the whole track as picture. The track is shown in the experimental arena, just like you see it during acquisition (not reduced). You can export with or without background image. This depends on the *Show background* check box in the *Zone data* box.

Raw data

All processed parameters base on the raw data, which are the coordinates of the tracking points in time and space. The reference space is the original video signal. That means, the zero/zero coordinate lies in the upper left corner of the video image.

A 4:3 PAL resolution (European standard) delivers coordinates from 0/0 to 720/576 (lower right corner).
4:3 NTSC (American standard) is mapped by coordinates from 0/0 to 480

These are just examples and there are a lot of other possible resolutions in PAL and NTSC. Digital cameras and especially HD cameras can have completely different resolutions. But they are all supported by the *Viewer*.

Coordinates can be exported as list in Comma Separated Values (*.csv) or Excel (*.xlsx) format by operating ***Export raw data...***

Figure 102 gives an example for a circular tracking area placed in the video image. In this example
Y-coordinates from zero to 500 and
X-coordinates from 100 to 600 are possible.

Trim data

The gray area is the video signal.
The blue area is the tracking area.

for details please see chapter *Data Analysis*, page 49.

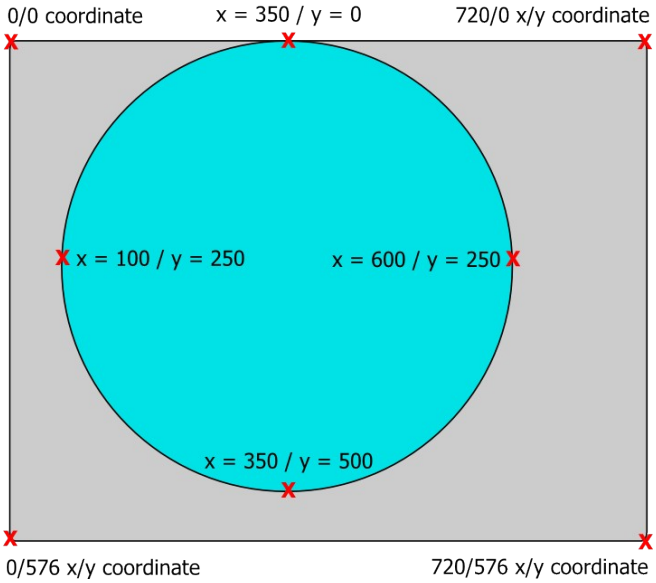


figure 102

Raw data:

Date	Time	Head	Body	Tail
26.01.2007	15:11:50	(63,124)	(76,109)	(99,88)
26.01.2007	15:11:50	(58,133)	(71,118)	(97,97)
26.01.2007	15:11:51	(70,133)	(71,118)	(95,94)
26.01.2007	15:11:51	(80,134)	(74,118)	(85,89)
26.01.2007	15:11:51	(92,139)	(78,125)	(72,97)
26.01.2007	15:11:51	(103,140)	(87,134)	(73,111)
26.01.2007	15:11:51	(115,145)	(97,140)	(73,120)
26.01.2007	15:11:52	(138,166)	(119,154)	(99,138)
26.01.2007	15:11:52	(157,182)	(139,171)	(116,148)
26.01.2007	15:11:49	(112,106)	(133,101)	(162,86)
26.01.2007	15:11:49	(87,95)	(108,98)	(137,98)
26.01.2007	15:11:50	(98,96)	(90,92)	(81,89)
26.01.2007	15:11:50	(81,97)	(86,92)	(93,99)
26.01.2007	15:11:50	(74,113)	(86,97)	(116,89)

figure 103

Data Replay

From the *Data replay* menu (see also page 51) you can export the ethogram as BMP image file and the *Grid* (figure 91) as CSV or Excel file. Switch between *Grid* and *Chart* view with the tabs in figure 90 element 3.

figure 104

Viewer plug-ins

Plug-ins upgrade the *Viewer* tracking software. The idea is to keep the core of the software as simple as possible and to outsource features into interfaces you can switch on and off. Most plug-ins deliver additional control features (*Database* connection or *Remote Control*) and/or test specific parameter analysis. If you need Y maze parameters, you can switch on the *Y Maze Plug-In*. If you want to run Morris water maze tests, there is a plug-in for the relevant parameters too.

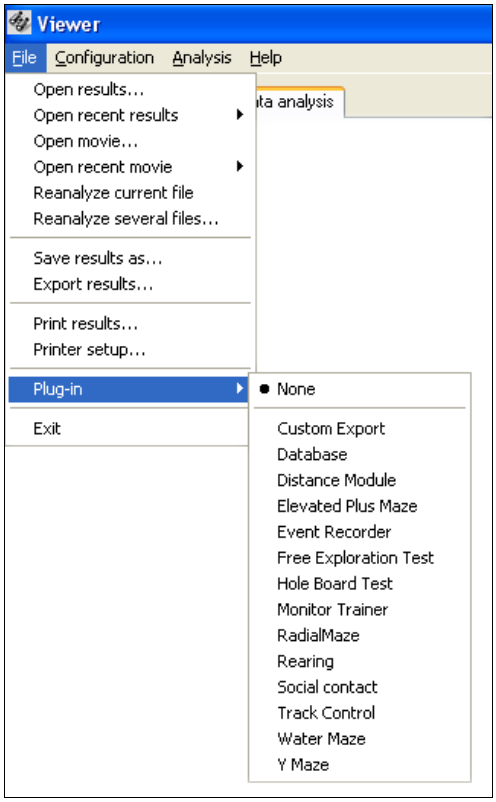


figure 105

Similar to the *Viewer* interfaces plug-in results and plug-in configuration items are placed on tabbed pages. When a plug-in is activated pages are added to the software interface Figure 104 shows an example with *Hole Board Test* tabs.
On the *Configuration* tab settings are implemented to make the plug-in work as desired. For example parameter thresholds, definition of target zones (*Watermaze*, *Barnes Maze*, *Hole Board*), external device control (doors, hydraulic platform etc.), automated result file saving.
The plug-in main tab contains results, statistics and visualizations.

Plug-ins have to be understood as autonomous pieces of software that drain or feed data from or to the *Viewer* system. Figure 106 shows different ways of information flow in a test specific analyzing plug-in.

Note: It is very important to ensure that the plug-in is informed about how *Viewer* is configured. It retrieves these pieces of information automatically whenever a *Viewer* configuration is loaded. This happens automatically whenever *Viewer* is started (the software, not acquisition) and whenever a result file is loaded.
It does not recognize when the *Viewer* configuration is changed but not saved and reloaded!

While *Viewer* is running plug-ins can be switched on and off at will from the *File* menu (see figure 105). Because they are completely processed in the main memory when loaded, it is recommended to switched all not used plug-ins off to spare memory capacity. Please read the *Plug-in Manager* chapter carefully to learn how to administer plug-ins best.

At the end of this document you will find a list of available plug-ins.

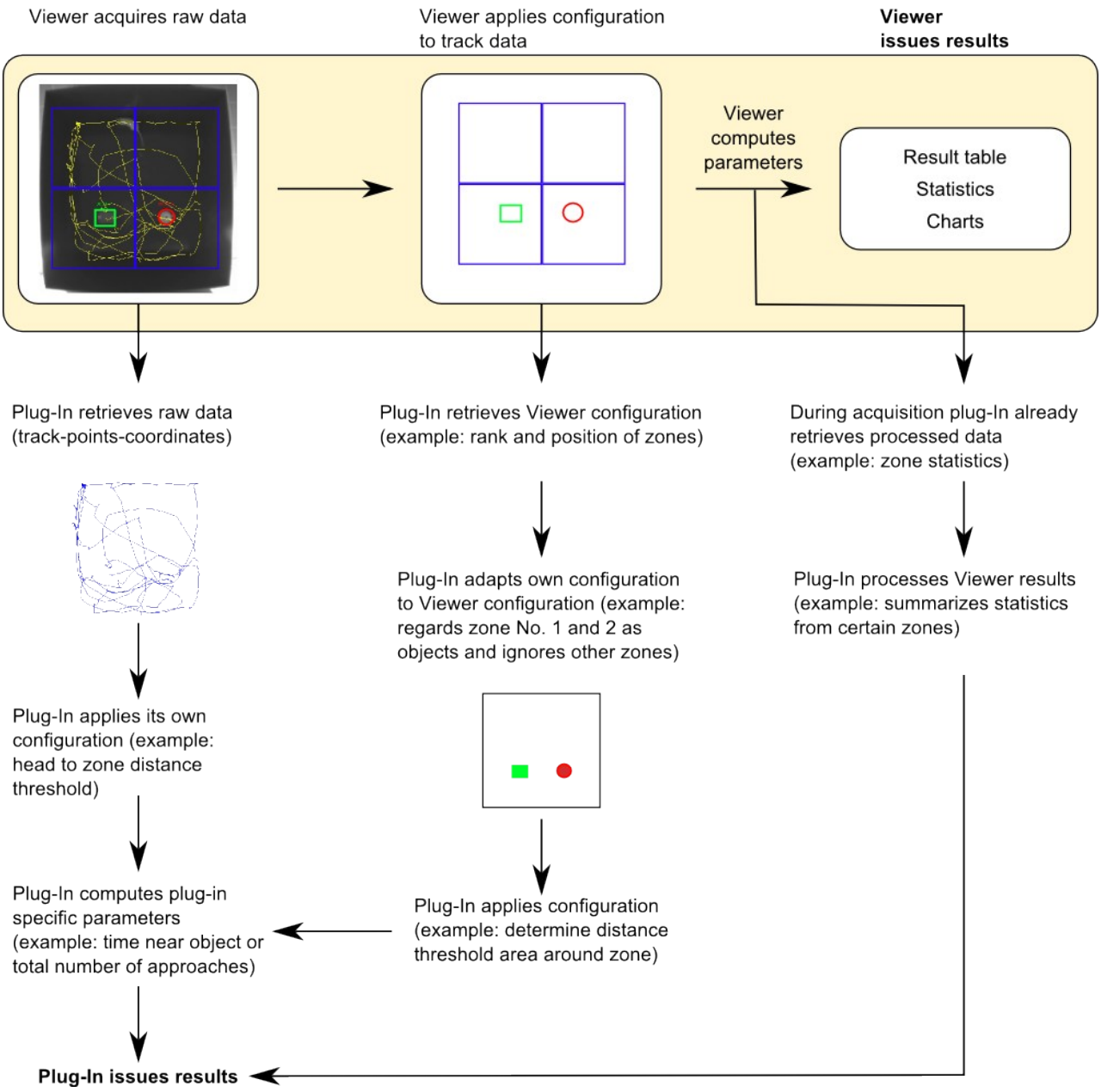

Viewer acquires raw data
Viewer applies configuration to track data
Viewer issues results
Viewer computes parameters
Result table
Statistics
Charts
Plug-In retrieves raw data (track-points-coordinates)
Plug-In retrieves Viewer configuration (example: rank and position of zones)
During acquisition plug-In already retrieves processed data (example: zone statistics)
Plug-In adapts own configuration to Viewer configuration (example: regards zone No. 1 and 2 as objects and ignores other zones)
Plug-In processes Viewer results (example: summarizes statistics from certain zones)
Plug-In applies its own configuration (example: head to zone distance threshold)
Plug-In computes plug-in specific parameters (example: time near object or total number of approaches)
Plug-In applies configuration (example: determine distance threshold area around zone)
Plug-In issues results

figure 106

How to upgrade Viewer

To install a plug-in, just copy the DLL file (plug-in-name.dll) to the *Modules* folder in the *Viewer* main directory (figure 107). In case an existing plug in is updated, MS Windows asks if you want to overwrite the existing DLL file. Confirm the dialog with the *Yes* button – that's all.
Plug-in activation: Start *Viewer* and select the installed plug-in from the *Viewer* – *File* menu.
A second choice from the *File* menu replaces the currently active plug-in.
If more than one plug-in has to be integrated into your *Viewer*, you have to use the *Plug-In Manager* to define active and inactive plug-ins. (see next chapter).

File administration

When the *Viewer* starts with a new DLL file in the *Modules* folder, the plug-in creates its own directories. The path to the directory is different under Windows XP and Windows 7. But it always ends in

.../documents/BIOBSERVE/Viewer/Modules/Plug-in name/

One general configuration file (main.ini) and personal configuration files are saved there. Furthermore exported image files, tables and plug-in specific result files.
Most analyzing plug-ins like the *Radial Maze Plug-In* offer result pools with several experiments results in one file (one table).
You can define another target directory for all these files. But the open and save dialogs will always refer to these folders. And if you are not an Administrator of the computer, Windows 7 might not allow saving in other directories.

Note: The configuration file you create for your individual setup of main-program parameters (name.vcf), such as camera settings, is saved in the *Viewer - Config* folder and must not be confused with the plug-in main.ini.

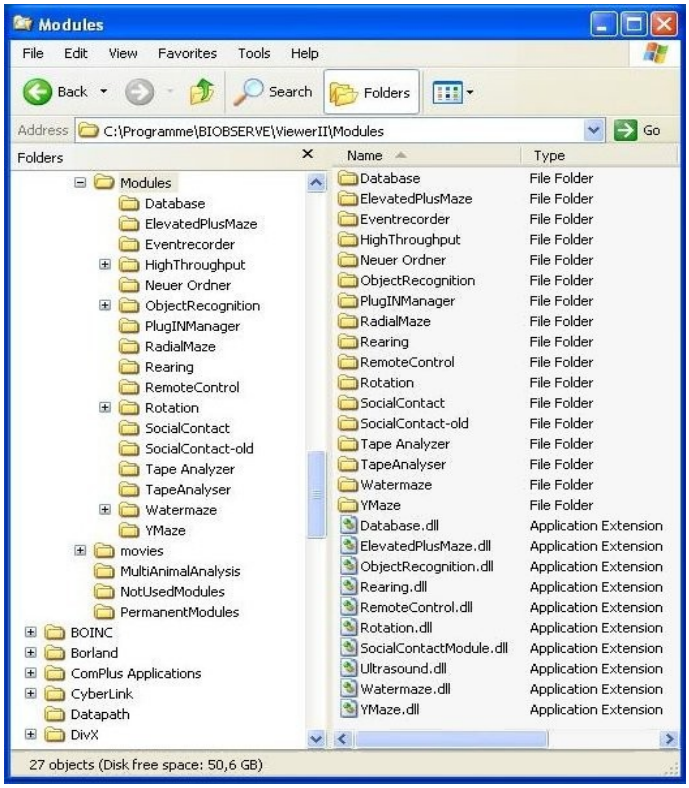


figure 107

Plug-In Manager

Plug-In Manager monitors your DLL files and changes their location in the *Modules* folders (see table below). The location of a plug-in file in one of these folder determines its status in the *Viewer.*

Folder	Status	how it is handled
NotUsedModules	*Invisible*	The plug-in is not loaded on *Viewer* start and does not appear in the *Plug-in* list in the *Viewer File* menu (figure Fehler: Referenz nicht gefunden).
Modules	*On request*	The plug-in is loaded on *Viewer* start and it can be selected from the *Plug-in* list in the *File* menu. Only one Plug-in from from the *File* menu can be activated.
PermanentModules	*Permanent*	The plug-in is loaded and activated automatically when *Viewer* is started. The plug-in can not be deactivated while *Viewer* is working. Plug-ins that have to run simultaneously have to be set to *Permanent* status.

More interesting than the buttons is the table (figure 108). *Plug-In-Manager* helps to set up *Viewer* for your needs at daily work. That's especially useful, if you have to switch between plug-ins every now and then.
The outer left column of the table contains a list of all plug-ins found in one of the three Modules folders (see table above). In the next three columns a status is defined for each plug-in. Click into a table field to change a status. Perform a single left mouse-click.
At least, there are some plug-ins that can not run simultaneously. A note will be displayed in the *Additional information* field, if incompatible plug-ins are activated.

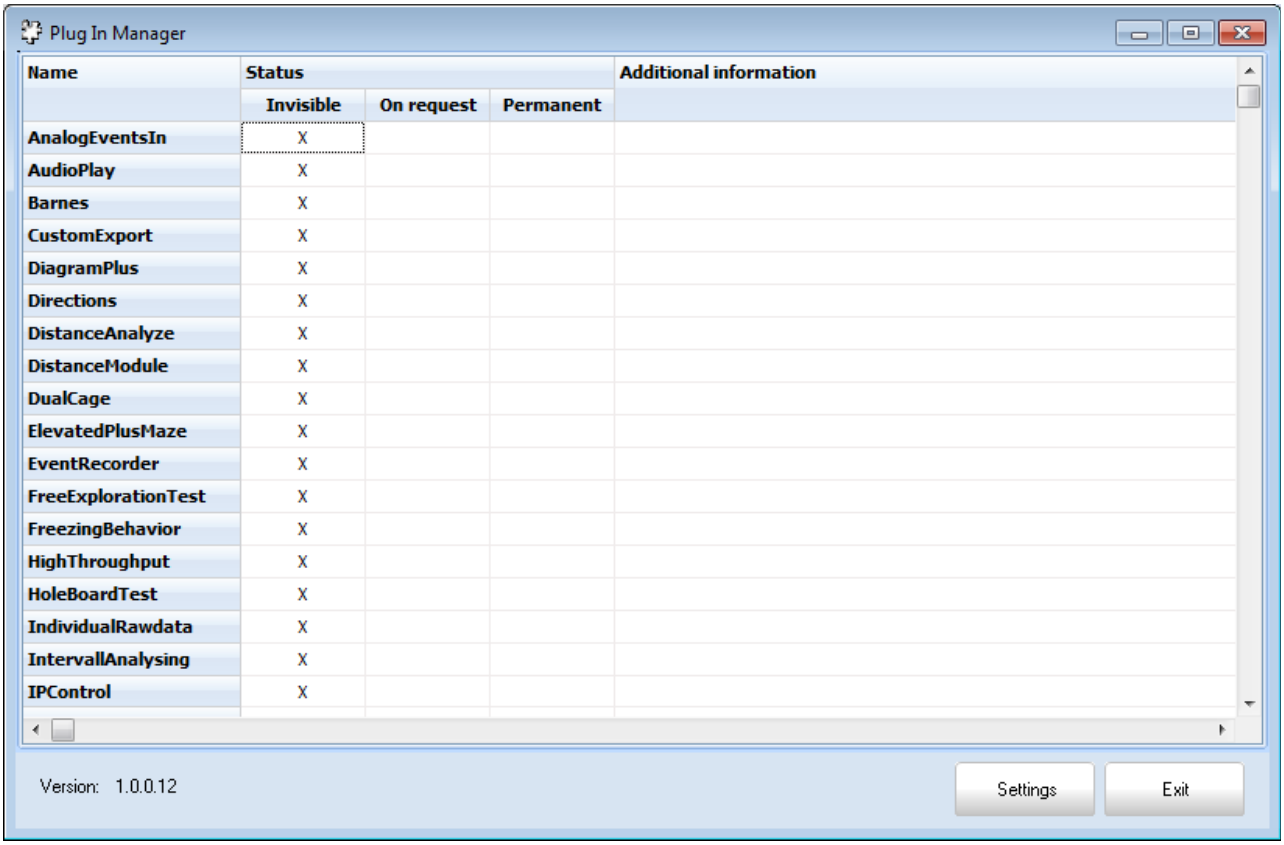


figure 108

The PlugInManager.exe file is delivered with your first plug-in purchased. It has to be placed in the Viewer main directory. The Exit button closes the software window. The Settings button opens a dialog where you have to determine the path to the directory where the plug-in folders are. In most cases this is the Viewer main directory. But that depends on the Microsoft Windows version. This has to be done only once. BIOBSERVE staff will help you to install everything correctly.

Troubleshooting:

- Do not use *Plug-In Manager* while *Viewer* is open! While *Viewer* is operating, it accesses the plug- in files. In a MS Windows system it is impossible to delete a currently used files. But this is, what *Plug-in Manager* must do to move the files from one folder to another.
  Windows XP will prompt an error message in this case. Windows 7 will produce a copy of the file without error message. But this is worse, because the plug-in will not work correctly with two copies next time.

Available plug-ins for your Viewer software

Custom Export	creates user-defined export files of your data
Database	collects information on a test series from an external database to automate result file saving and provides series analysis
Distance Module	computes distances between an animal and several zones within the tracking-arena
Elevated Plus Maze	registers durations of stay, latencies, number of entries and other test-specific parameters of an animal in an elevated plus maze arena
Eventrecorder	a useful tool to log up to 25 self defined behaviors by keyboard shortcuts
Free Exploration Test	for examining an animal's novelty/anxiety behavior concerning the boxes of the free exploration test or open box test
Freezing	detects freezing behavior in rats and mice according to user defined thresholds and filters
High Throughput	enables the Viewer to batch-analyze a great number of videos with no need to attend it
Hole Board Test	measures hole examinations with light beams, locates the examination by video tracking and delivers advanced statistics to describe memory performance
Monitor Trainer	for animal conditioning by case sensitive video display on several monitors
NeuroExplorer Export	exports selected data for Nex NeuroExplorer import
Object Recognition	delivers distances to an object's margin, detects sniffing behavior and analyzes the animals body axis alignment towards objects
Open Field	Produces experiment result pools with typical Open Field parameters
Radial Maze	records strategy and time parameters of animals in a radial maze test. The plug-in includes a session planner and a subject validation function
Rearing	detects and localizes vertical movements of animals by combined use of video cameras and photoelectric barriers.
Remote control	makes the Viewer sensitive for remote control signals. If the keyboard is out of reach and no colleague is available to put the animal in the arena, you can still start and stop acquisition in the very best moment.
Rotation	first tool offering user adjusted detection of count, speed and angle of rotation behavior in mice and rats
Social Contact	registers social behavior automatically (for example contacts, active vs. passive contacts and aggressive vs. submissive contacts)
Y Maze	registers durations of stay, latencies, number of entries and other test-specific parameters of an animal in an elevated Y maze arena

New plug-ins

Find a complete list on
http://www.biobserve.com/products/viewer/plugins/index.html

To request a new development contact viewer@biobserve.com

Thank you for choosing our software

Don't hesitate to contact us for help and consultations

www.biobserve.com